LC-NMR Accessory

Installation

Pub. No. 01-999036-00, Rev. C0800

VARIAN

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Pub. No. 01-999036-00, Rev. C0800

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Applicability of manual:
LC-NMR Accessory for UNITY*INOVA*, UNITY*plus*, and UNITY NMR spectrometer systems

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SAFETY PRECAUTIONS

The following warning and caution notices illustrate the style used in Varian manuals for safety precaution notices and explain when each type is used:

WARNING: ***Warnings*** **are used when failure to observe instructions or precautions could result in injury or death to humans or animals, or significant property damage.**

CAUTION: ***Cautions*** **are used when failure to observe instructions could result in serious damage to equipment or loss of data.**

Warning Notices

Observe the following precautions during installation, operation, maintenance, and repair of the instrument. Failure to comply with these warnings, or with specific warnings elsewhere in Varian manuals, violates safety standards of design, manufacturing, and intended use of the instrument. Varian assumes no liability for customer failure to comply with these precautions.

WARNING: **Persons with implanted or attached medical devices such as pacemakers and prosthetic parts must remain outside the 5-gauss perimeter from the centerline of the magnet.**

The superconducting magnet system generates strong magnetic fields that can affect operation of some cardiac pacemakers or harm implanted or attached devices such as prosthetic parts and metal blood vessel clips and clamps.

Pacemaker wearers should consult the user manual provided by the pacemaker manufacturer or contact the pacemaker manufacturer to determine the effect on a specific pacemaker. Pacemaker wearers should also always notify their physician and discuss the health risks of being in proximity to magnetic fields. Wearers of metal prosthetics and implants should contact their physician to determine if a danger exists.

Refer to the manuals supplied with the magnet for the size of a typical 5-gauss stray field. This gauss level should be checked after the magnet is installed.

WARNING: **Keep metal objects outside the 10-gauss perimeter from the centerline of the magnet.**

The strong magnetic field surrounding the magnet attracts objects containing steel, iron, or other ferromagnetic materials, which includes most ordinary tools, electronic equipment, compressed gas cylinders, steel chairs, and steel carts. Unless restrained, such objects can suddenly fly towards the magnet, causing possible personal injury and extensive damage to the probe, dewar, and superconducting solenoid. The greater the mass of the object, the more the magnet attracts the object.

Only nonferromagnetic materials—plastics, aluminum, wood, nonmagnetic stainless steel, etc.—should be used in the area around the magnet. If an object is stuck to the magnet surface and cannot easily be removed by hand, contact Varian service for assistance.

Warning Notices (*continued*)

Refer to the manuals supplied with the magnet for the size of a typical 10-gauss stray field. This gauss level should be checked after the magnet is installed.

WARNING: **Only qualified maintenance personnel shall remove equipment covers or make internal adjustments.**

Dangerous high voltages that can kill or injure exist inside the instrument. Before working inside a cabinet, turn off the main system power switch located on the back of the console, then disconnect the ac power cord.

WARNING: **Do not substitute parts or modify the instrument.**

Any unauthorized modification could injure personnel or damage equipment and potentially terminate the warranty agreements and/or service contract. Written authorization approved by a Varian, Inc. product manager is required to implement any changes to the hardware of a Varian NMR spectrometer. Maintain safety features by referring system service to a Varian service office.

WARNING: **Do not operate in the presence of flammable gases or fumes.**

Operation with flammable gases or fumes present creates the risk of injury or death from toxic fumes, explosion, or fire.

WARNING: **Leave area immediately in the event of a magnet quench.**

If the magnet dewar should quench (sudden appearance of gasses from the top of the dewar), leave the area immediately. Sudden release of helium or nitrogen gases can rapidly displace oxygen in an enclosed space creating a possibility of asphyxiation. Do not return until the oxygen level returns to normal.

WARNING: **Avoid liquid helium or nitrogen contact with any part of the body.**

In contact with the body, liquid helium and nitrogen can cause an injury similar to a burn. Never place your head over the helium and nitrogen exit tubes on top of the magnet. If liquid helium or nitrogen contacts the body, seek immediate medical attention, especially if the skin is blistered or the eyes are affected.

WARNING: **Do not look down the upper barrel.**

Unless the probe is removed from the magnet, never look down the upper barrel. You could be injured by the sample tube as it ejects pneumatically from the probe.

WARNING: **Do not exceed the boiling or freezing point of a sample during variable temperature experiments.**

A sample tube subjected to a change in temperature can build up excessive pressure, which can break the sample tube glass and cause injury by flying glass and toxic materials. To avoid this hazard, establish the freezing and boiling point of a sample before doing a variable temperature experiment.

Warning Notices (*continued*)

WARNING: **Support the magnet and prevent it from tipping over.**

The magnet dewar has a high center of gravity and could tip over in an earthquake or after being struck by a large object, injuring personnel and causing sudden, dangerous release of nitrogen and helium gasses from the dewar. Therefore, the magnet must be supported by at least one of two methods: with ropes suspended from the ceiling or with the antivibration legs bolted to the floor. Refer to the *Installation Planning Manual* for details.

WARNING: **Do not remove the relief valves on the vent tubes.**

The relief valves prevent air from entering the nitrogen and helium vent tubes. Air that enters the magnet contains moisture that can freeze, causing blockage of the vent tubes and possibly extensive damage to the magnet. It could also cause a sudden dangerous release of nitrogen and helium gases from the dewar. Except when transferring nitrogen or helium, be certain that the relief valves are secured on the vent tubes.

WARNING: **On magnets with removable quench tubes, keep the tubes in place except during helium servicing.**

On Varian 200- and 300-MHz 54-mm magnets only, the dewar includes removable helium vent tubes. If the magnet dewar should quench (sudden appearance of gases from the top of the dewar) and the vent tubes are not in place, the helium gas would be partially vented sideways, possibly injuring the skin and eyes of personnel beside the magnet. During helium servicing, when the tubes must be removed, carefully follow the instructions and safety precautions given in the manual supplied with the magnet.

Caution Notices

Observe the following precautions during installation, operation, maintenance, and repair of the instrument. Failure to comply with these cautions, or with specific cautions elsewhere in Varian manuals, violates safety standards of design, manufacturing, and intended use of the instrument. Varian assumes no liability for customer failure to comply with these precautions.

CAUTION: **Keep magnetic media, ATM and credit cards, and watches outside the 5-gauss perimeter from the centerline of the magnet.**

The strong magnetic field surrounding a superconducting magnet can erase magnetic media such as floppy disks and tapes. The field can also damage the strip of magnetic media found on credit cards, automatic teller machine (ATM) cards, and similar plastic cards. Many wrist and pocket watches are also susceptible to damage from intense magnetism.

Refer to the manuals supplied with the magnet for the size of a typical 5-gauss stray field. This gauss level should be checked after the magnet is installed.

Caution Notices (*continued*)

CAUTION: **Keep the PCs, (including the LC STAR workstation) beyond the 5-gauss perimeter of the magnet.**

Avoid equipment damage or data loss by keeping PCs (including the LC workstation PC) well away from the magnet. Generally, keep the PC beyond the 5-gauss perimeter of the magnet. Refer to the *Installation Planning Guide* for magnet field plots.

CAUTION: **Check helium and nitrogen gas flowmeters daily.**

Record the readings to establish the operating level. The readings will vary somewhat because of changes in barometric pressure from weather fronts. If the readings for either gas should change abruptly, contact qualified maintenance personnel. Failure to correct the cause of abnormal readings could result in extensive equipment damage.

CAUTION: **Never operate solids high-power amplifiers with liquids probes.**

On systems with solids high-power amplifiers, never operate the amplifiers with a liquids probe. The high power available from these amplifiers will destroy liquids probes. Use the appropriate high-power probe with the high-power amplifier.

CAUTION: **Take electrostatic discharge (ESD) precautions to avoid damage to sensitive electronic components.**

Wear a grounded antistatic wristband or equivalent before touching any parts inside the doors and covers of the spectrometer system. Also, take ESD precautions when working near the exposed cable connectors on the back of the console.

Radio-Frequency Emission Regulations

The covers on the instrument form a barrier to radio-frequency (rf) energy. Removing any of the covers or modifying the instrument may lead to increased susceptibility to rf interference within the instrument and may increase the rf energy transmitted by the instrument in violation of regulations covering rf emissions. It is the operator's responsibility to maintain the instrument in a condition that does not violate rf emission requirements.

Chapter 1. Introduction

Sections in this chapter:

- 1.1 “About This Manual” this page
- 1.2 “Compatibility” page 12
- 1.3 “Preinstallation Requirements” page 13
- 1.4 “Site Layout Guidelines” page 14
- 1.5 “Preparing Solvents” page 18
- 1.6 “Caring for the HPLC System and Microflow Probe” page 18

The Varian LC-NMR accessory combines the separation capabilities of high-performance liquid chromatography (HPLC) with the structure elucidation capabilities of NMR. The HPLC and NMR systems are each complete and can be operated individually. LC-NMR integrates the two systems into a new class of analytical instrument. Chromatography is an analytical technique that, using the right conditions, separates individual components from a mixture.

The LC-NMR accessory includes the following components (See Figure 1):

- Microflow probe.
- Complete HPLC system, including pump, variable-wavelength UV detector, PC, and column, a peak sense module.
- LC-NMR software, added to VNMR, which includes a variety of pulse sequences, pulse shapes, and macros for LC-NMR data acquisition and data processing.

1.1 About This Manual

The installation instructions in this manual primarily cover the integration of the HPLC system with the NMR spectrometer. The major components—including the LC detector, LC pump, and valves—of the HPLC system should be set up according to the *Varian Chromatography Systems* manuals. The NMR spectrometer should be installed and running properly before being integrated with the HPLC system. The first three chapters of this manual provide instructions for integrating the two systems.

1.2 Compatibility

The LC-NMR accessory is fully compatible with UNITY*INOVA*, UNITY*plus*, and UNITY NMR spectrometer systems with a Microflow probe.

WARNING: **Keep metal objects, including tools, electronic equipment, compressed gas cylinders, steel chairs, and steel carts, away from the magnet. Unless restrained, such objects can suddenly fly towards the magnet, causing personal injury and extensive damage to the probe, the dewar, and the superconducting solenoid. Only nonferromagnetic materials (such as plastics, aluminum, wood, and stainless steel) should be used in the area around the magnet dewar**

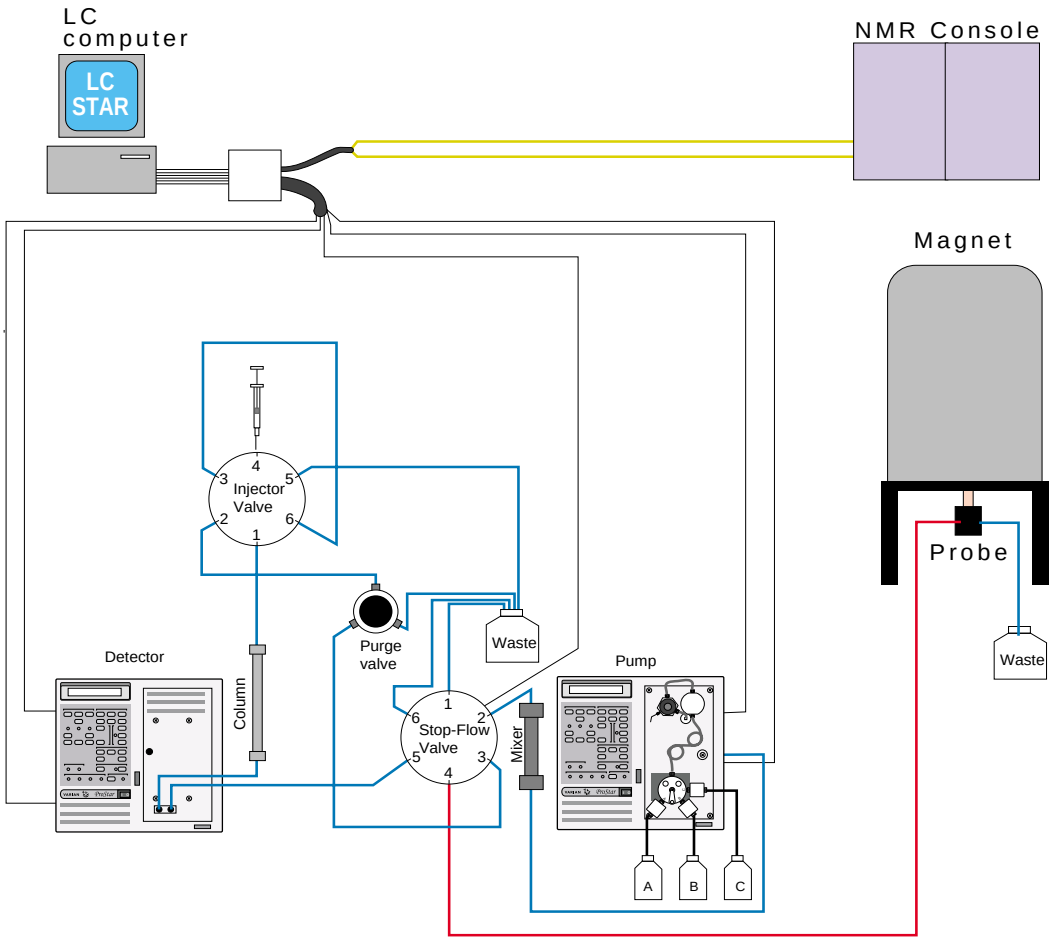


Figure 1. LC-NMR Block Diagram

1.3 Preinstallation Requirements

This section lists the items needed for the LC-NMR accessory installation. Everything listed in this section must be in place before you begin the installation.

Equipment, Samples, and Solvents

You must have the following equipment, samples, and solvents:

- A nonmagnetic table, at least 0.9 m by 1.2 m (3 ft by 4 ft), to hold the LC hardware.
- Either gas cylinder of helium, outfitted with 0.25-inch OD Teflon tubing with aerator (this will be used for sparging the acetonitrile solvent), or the necessary degassing equipment. Do not use an aspirator.
- At least 300 mL of D_2O, 99.8 atom percent or better.
- At least 100 mL of acetone-d_6.
- At least 300 mL of OmniSolv brand acetonitrile (EM Science part no. AX0142-l).
- Research grade sucrose.
- At least three, 100-mL volumetric flasks for preparing standard samples.
- An appropriate waste container for HPLC effluent. The cover of the container must have a hole in the top of about 2 mm diameter. The hole is used for the outlet of the LC-NMR Microflow probe.
- Air supply for the pneumatic valve.

Site

Your site must have the following:

- Five, 110 Vac or 220 Vac outlets within 2m (6 ft) of where the table holding the LC hardware will be located. Several extension cords should be available for the installation.
- Approval by Varian of the proposed layout of the LC-NMR lab.

Personnel

A person experienced in liquid chromatography must be present during the installation. Having this person on site ensures that vital information is shared regarding the following:

- The performance of the HPLC system.
- The interface between the LC system and the NMR spectrometer.
- The isolation of the LC system from the NMR spectrometer.
- The overall operation of the LC-NMR accessory.

At least one NMR spectroscopist must be present during the installation, calibration, and system tests.

1.4 Site Layout Guidelines

This section provides guidelines for arranging an LC-NMR lab. The illustrations in this section provide a room layout diagrams for you to use as a guideline for setting up your LC-NMR room.

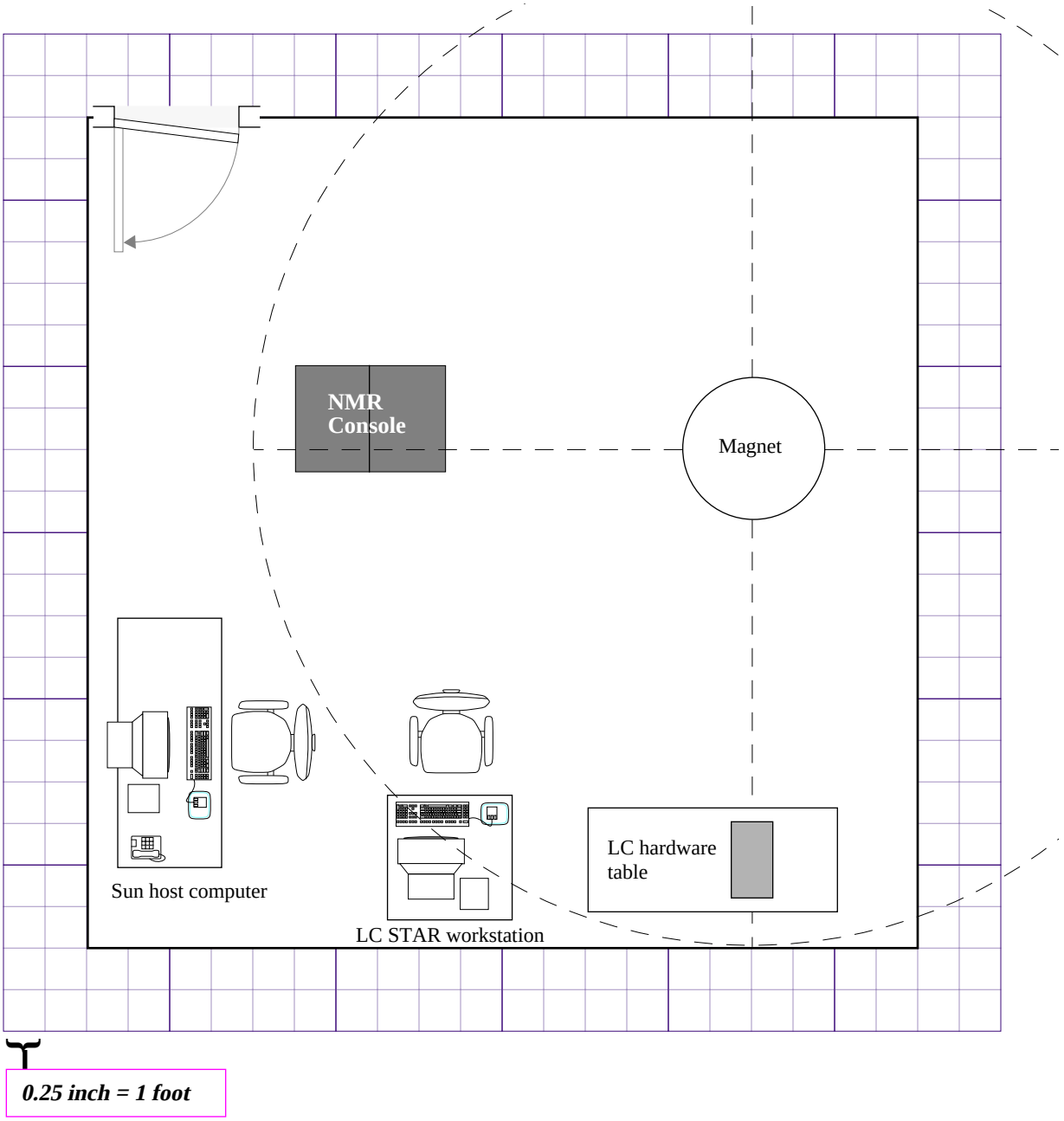
The following room layouts are illustrated:

- "LC-NMR Minimum Room Layout" on page 15
- "LC-NMR Suggested Room Layout 1" on page 16
- "LC-NMR Suggested Room Layout 2" on page 17

Consider the following when developing a room layout:

- Stray magnetic fringe fields. The *Installation Planning Guide* that came with your system contains magnetic field plots.
- The distance between the NMR console and the magnet must be less than 3 m (10 ft) to accommodate cable lengths between the console and the preamplifier housing (magnet leg).
- The transfer tube between the LC system and the probe is 4.6 m (15 ft), no longer. Therefore, the distance between the LC hardware and the magnet should be about 3 m (10 ft) to accommodate drops and rises.
- The room must provide access for cryogen servicing of the magnet. For cryogen servicing, cryogen dewars need to be moved into the room, near the magnet.
- The LC STAR workstation (PC) should be set up close to the Sun host computers.
- Cable lengths from the LC STAR workstation (PC)—up to 3.35 m (11 ft) between the LC workstation and the LC hardware; up to 12.2 m (40 ft) between the LC workstation and the NMR console.
- Cable lengths from the Sun workstation and the NMR console—up to 24.4 m (80 ft).

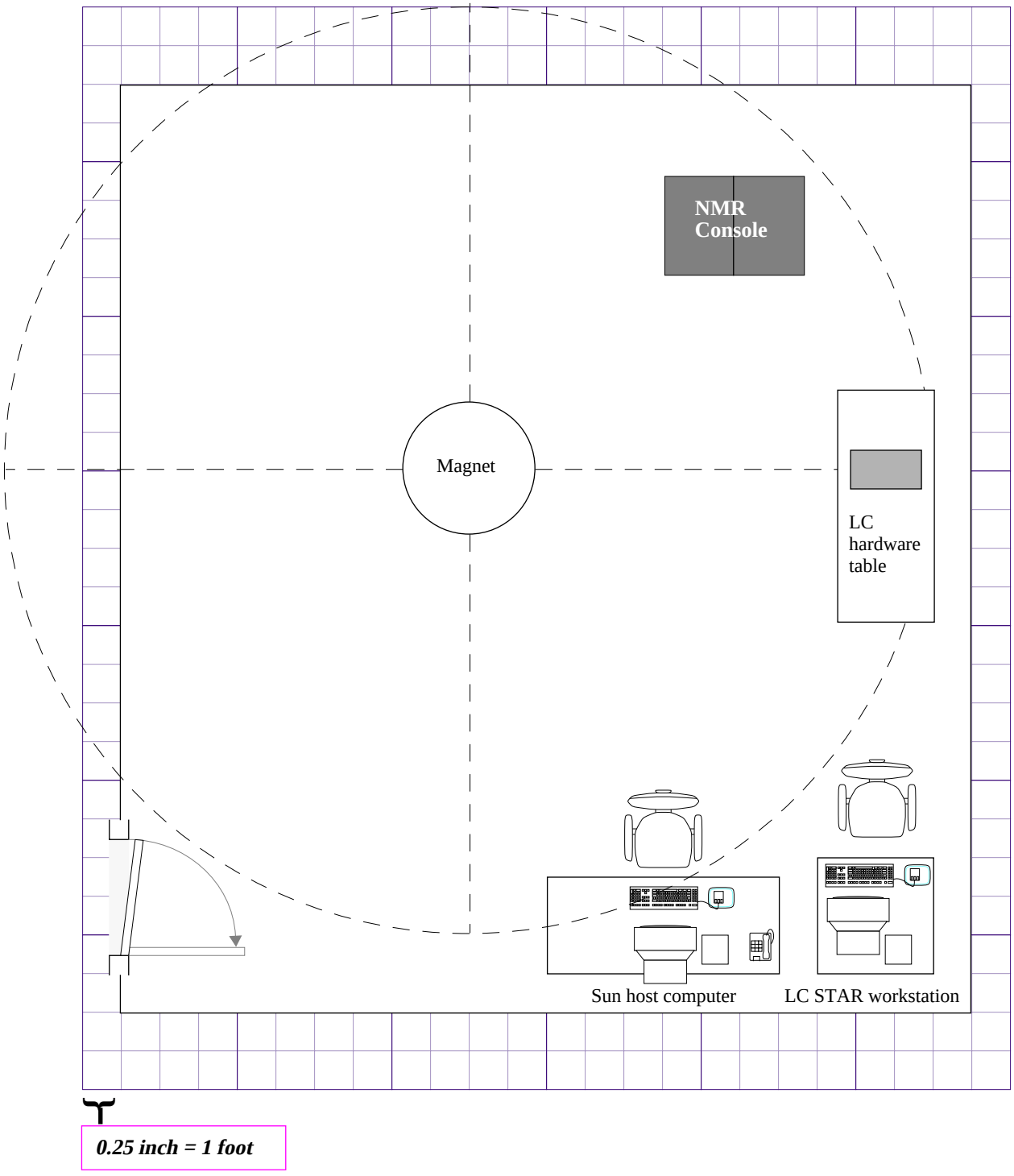
LC-NMR Minimum Room Layout



Room dimensions are about 6.1 m × 6.1 m (20 ft × 20 ft). The standard NMR cabinet is 111 cm × 78 cm (44 in. × 31 in.). Typical table size is 183 cm × 76 cm (72 in. × 30 in.). The magnet is 97.8 cm (38.5 in.) in diameter.

Refer to the *Installation Planning Guide* for component dimensions. Drawn approximately to scale.

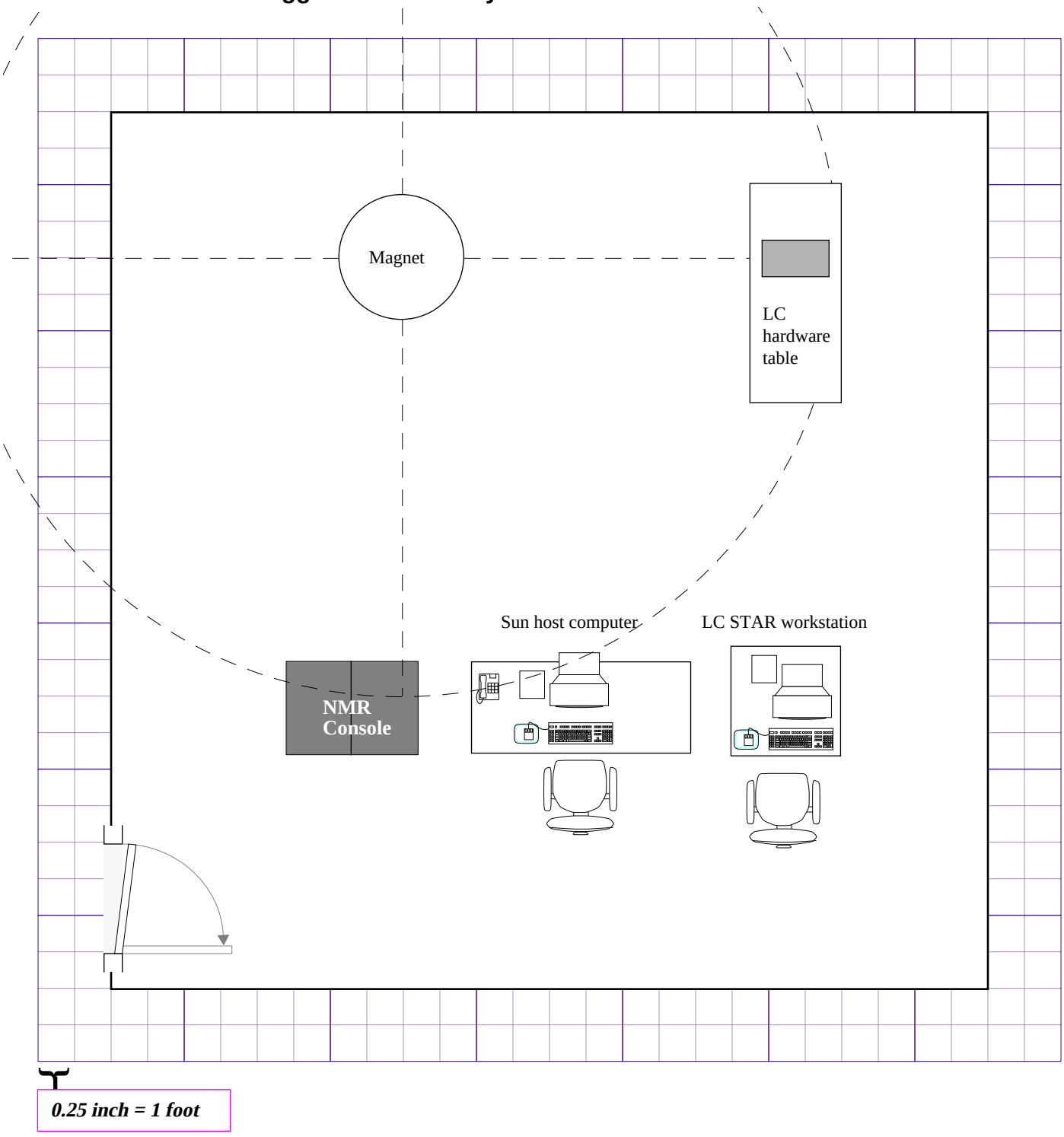
LC-NMR Suggested Room Layout 1



Room dimensions are about 6.7 m × 7.3 m (22 ft × 24 ft). The standard NMR cabinet is 111 cm × 78 cm (44 in. × 31 in.). Typical table size is 183 cm × 76 cm (72 in. × 30 in.). The magnet is 97.8 cm (38.5 in.) in diameter.

Refer to the *Installation Planning Guide* for component dimensions. Drawn approximately to scale.

LC-NMR Suggested Room Layout 2



Room dimensions are about 7.3 m × 7.3 m (24 ft × 24 ft). The standard NMR cabinet is 111 cm × 78 cm (44 in. × 31 in.). Typical table size is 183 cm × 76 cm (72 in. × 30 in.). The magnet is 97.8 cm (38.5 in.) in diameter.

Refer to the *Installation Planning Guide* for component dimensions. Drawn approximately to scale.

1.5 Preparing Solvents

This section provides information to consider when preparing solvents for LC-NMR.

- De-gas or sparge solvents to prevent bubble formation in the probe cell.
- Filter solvents that have been prepared by mixing and/or dissolving one compound into another to remove residual or precipitated solid material.
- Cover solvent containers for the following reasons:
    - To prevent dust from accumulating
    - To prevent excessive exposure to vapors
    - To prevent pickup or exchange of D with H.
- Install a vent when the solvent container is connected to the HPLC system. This is especially important for solvents that are being continuously sparged.

1.6 Caring for the HPLC System and Microflow Probe

This section provides recommendations about caring for the HPLC system and the Microflow probe.

- Do not use solvents that are incompatible with PEEK, quartz, 316 stainless steel, tefzel, or teflon. Also, do not use solvents or buffers that are incompatible with the column's packing. For example, high pH buffer systems may dissolve silica in C-18 and C-8 columns.
- Do not allow solvent to drip onto the top of the LC pump, the LC detector, or any other electronic device where the solvent can penetrate the cabinet and reach the circuitry.
- If you are using buffers, flush the system with D_2O or water daily to dissolve any salts that might have precipitated.
- Do not leave buffers or only water in the HPLC system or Microflow probe. Bacteria can grow and block tubing and the column. Always rinse the HPLC system and the Microflow probe with D_2O or water first, followed by a mixture of at least 25% organic solvent and D_2O or water.
- To prevent the probe from drying out between use, make sure that the probe inlet and outlet ports are sealed with a piece of tubing connected between them or with no-hole ferrules connected to each port. Similarly, seal the 310 detector if it will not be used for a while.
- Periodically flush the probe with the strong solvent you are using to remove any compounds that may adhere to the probe flow cell.
- Do not connect tubing with less than a 0.010-inch internal diameter (I.D.) to the probe outlet (larger I.D.s are preferred). Long runs of small I.D. tubing cause excessive pressure in the probe that can break seals and create leaks within the probe.
- Do not subject the probe to excessive mechanical vibrations or violent motion.

Chapter 2. HPLC Hardware Setup

Sections in this chapter:

- 2.1 “Assembling the Valves and ProStar LC Pump Plumbing” this page
- 2.2 “Setting Up the ProStar LC Detector” page 21
- 2.3 “Installing the Stop-Flow Valve and Plumbing” page 25
- 2.4 “Unpacking and Assembling the LC STAR Workstation” page 27
- 2.5 “Connecting the Stop-Flow Cable” page 28
- 2.6 “Installing the Powered Event Module” page 31
- 2.7 “Leak-Testing the System” page 34
- 2.8 “Using the LC Pump” page 36

Most of the HPLC hardware is set up using the instructions provided in *Varian Chromatography Systems* documentation. Detailed instructions are provided in this chapter for plumbing the pump and valves, connecting the detector, assembling the LC STAR workstation, and connecting the HPLC system to the NMR spectrometer. The NMR spectrometer must be installed and running properly before the HPLC system can be integrated with it. This chapter also provides some basic instructions for using the LC pump and detector.

If you are unfamiliar with chromatographs, and with Varian STAR Chromatography systems in particular, you may wish to read the *Varian Chromatography Systems* manuals before beginning the HPLC hardware setup.

The installation kit provides two rolls of PEEK tubing. The blue PEEK tubing (0.010-inch I.D.) is used to connect the various LC valves. The red PEEK tubing (0.005-inch I.D.) is used as the transfer line, to connect the probe to the LC system.

2.1 Assembling the Valves and ProStar LC Pump Plumbing

The injector valve, purge valve, column, valve mounting plate, mounting track, column mounting hardware, and thumbscrews are packaged in separate plastic bags in the universal valve mounting kit. This kit enables you to mount these valves and the column onto the side of the LC pump, which has holes tapped in both sides.

We suggest you mount the assembly on the left side of the pump to facilitate plumbing connections. Figure 2 illustrates how the universal valve mounting kit is assembled. Instructions in the Chromatography Systems manuals provide further information.

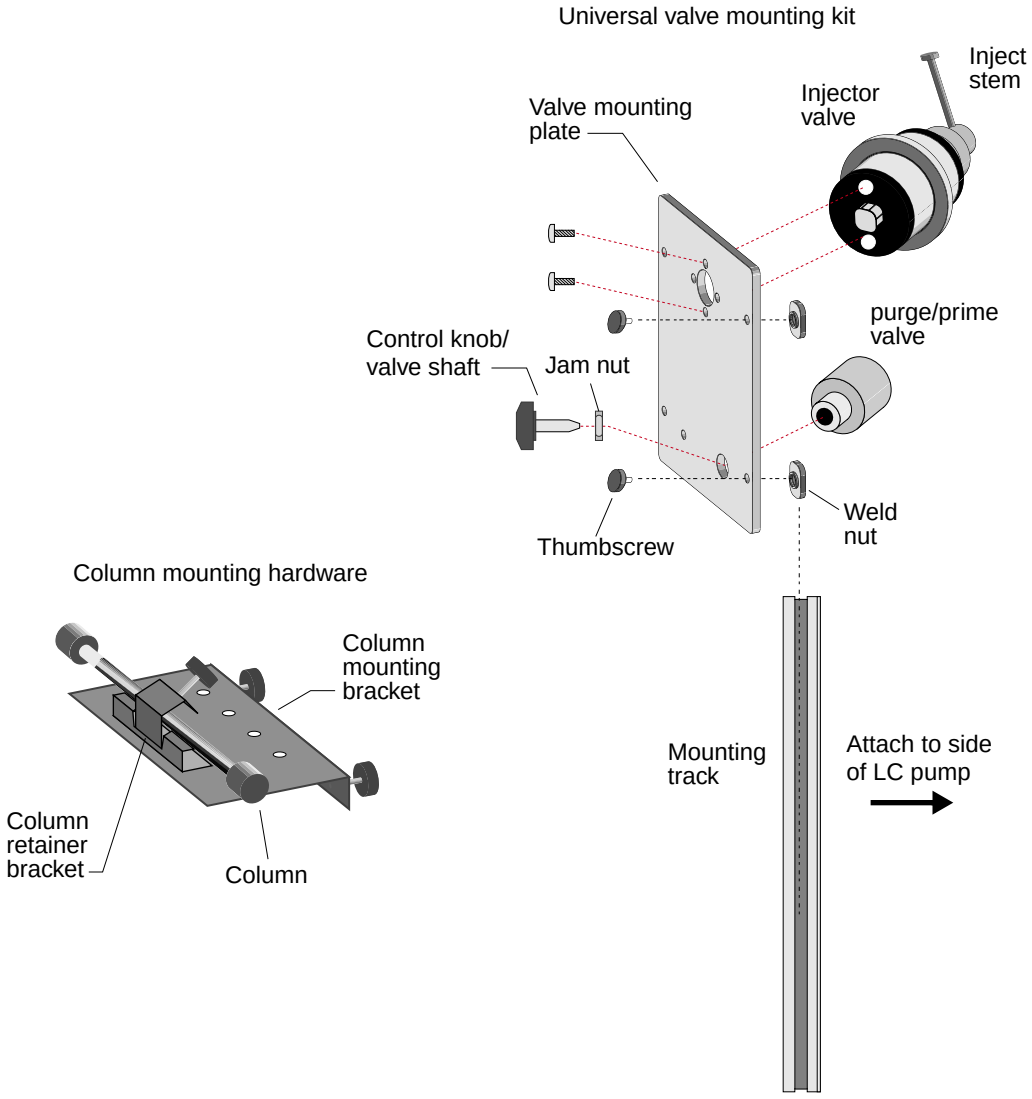


Figure 2. Mounting the Injector and Purge/Prime Valves

2.2 Setting Up the ProStar LC Detector

Use the instructions provided in the *Varian Chromatography Systems* manuals to unpack and set up the ProStar LC detector. Choose one of the procedures below to set up the *ProStar 310 detector* or the *ProStar 330 detector*.

Setting Up the ProStar 310 Detector

Set up the ProStar 310 detector as follows:

1. Connect a cable from J201 on the detector to J201 on the pump.
2. Make sure the J2 voltage switch on the back of the detector is set to 1 V, as shown in Figure 3.
3. Make sure the thumbwheel switch (S201 DEVICE ADDR) on the back of the LC detector is set to 3; if not, set it to 3, as shown in Figure 3.

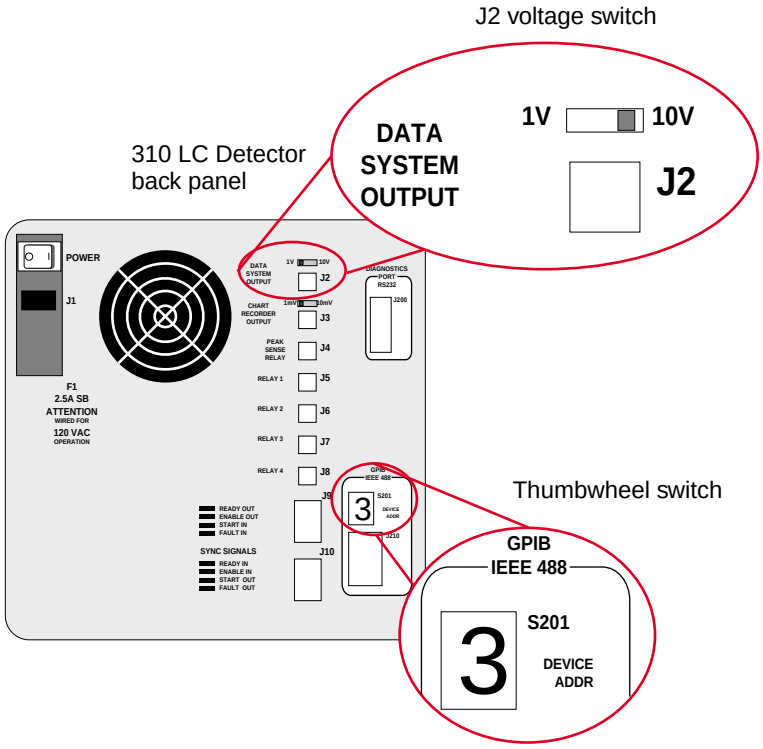
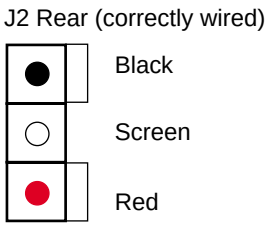


Figure 3. 310 Detector Voltage and Device Address Switches

4. Make sure the wiring on the J2 connector is configured as shown in the illustration on the right.

   The J2 connector is incorrectly wired if the stop-flow valve constantly rotates or the 16-port valve randomly increments. Make sure the wiring matches the picture to the right.



Setting Up the ProStar 330 Detector

Set up the ProStar 330 detector using the following procedures:

- Unpacking the Detector and Removing Shipping Supports
- Installing the Analog Output Board
- Installing the High-Pressure Flow Cell
- Loading the Control Software and Installing the ProStar 330 GPIB Card in the Dell Computer
- Loading Polyview Software
- Turning the Lamp On and Off

Unpacking the Detector and Removing Shipping Supports

1. Remove the two transport screws, as described by the instructions taped onto the cover of the instrument. Figure 4 shows the location of the two transport screws on the bottom of the ProStar 330 detector.

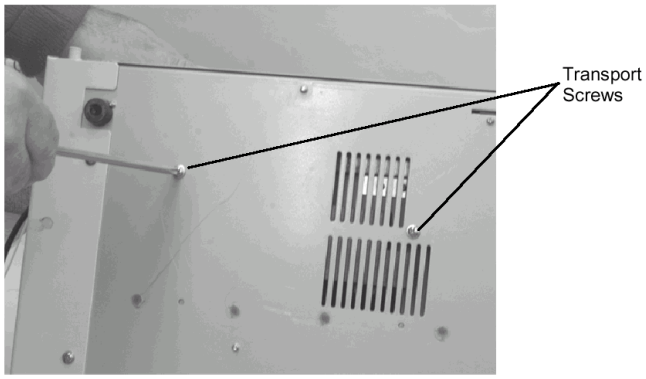


Figure 4. ProStar 330 Transport Screw Locations

2. Position the ProStar 330 on the bench and remove the side panel using the thumb screws.
3. Remove the flow cell support from around the flow cell by reaching in and removing the supporting material by hand, as shown in Figure 5.

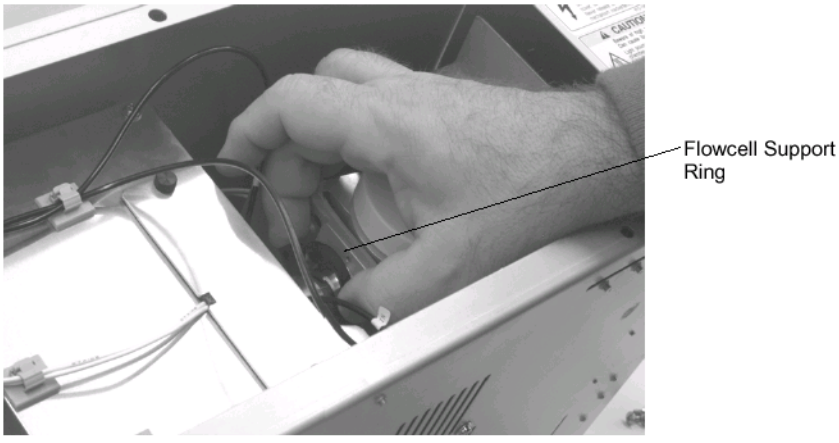


Figure 5. ProStar 330 Flow Cell Support Removal

4. Unscrew the thumbscrews from the Hg lamp. Remove the cover with the lamp and remove the sponge from the detector, as shown in Figure 6.

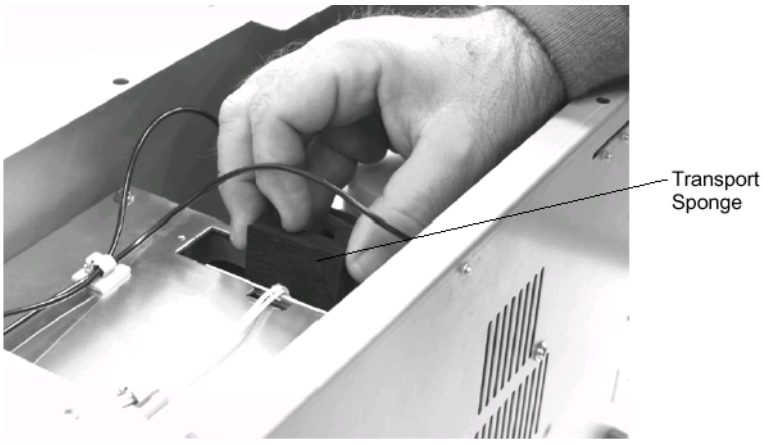


Figure 6. ProStar 330 Sponge Removal

5. If the modules are to be stacked, the ProStar 330 should be placed on top of the ProStar 230 plump.

Installing the Analog Output Board

Figure 7 shows the location of the Analog Output board.

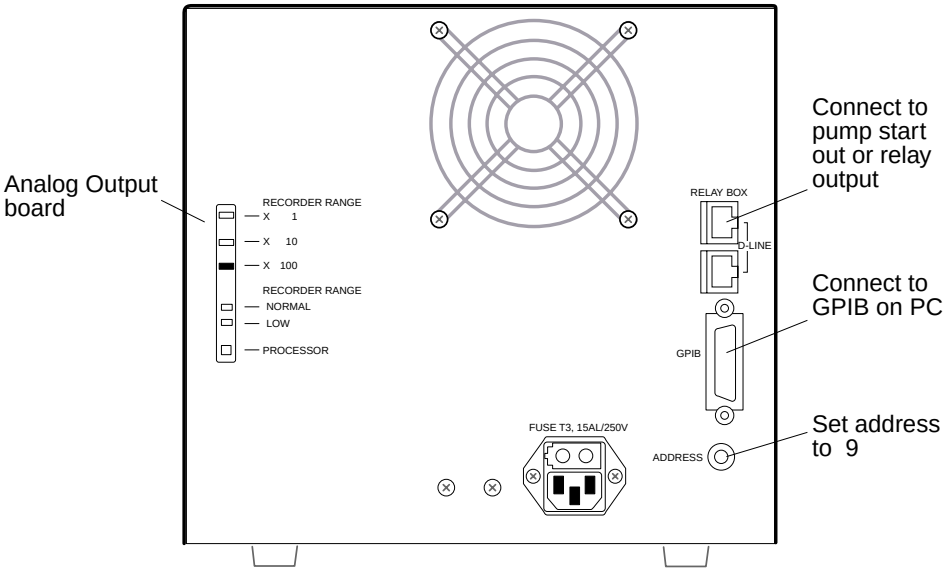


Figure 7. ProStar 330 Analog Output Board Install

1. Remove the blank panel from the left side on the back of the ProStar 330 by loosening the screws.
2. Install the Analog Output board.
3. Set the switch to normal for calibration purposes. The switch can be set to low for better detection of small peaks.

The x1, x10, and x100 settings are only used with the recorder output, which will not be accessed.

Installing the High-Pressure Flow Cell

Replace the low-pressure flow cell with the high-pressure flow cell.

1. Remove the low-pressure flow cell that was shipped with the system. Pull back the retaining ring and lift the flow cell out. Gently remove the attached tubing from the supports and pull the unit away from the box.
2. Install the high-pressure flow cell by pulling back the retaining ring and positioning the new flow cell in the light path. Put the attached tubing into the side support. Thread the inlet tubing out the inlet port and the outlet tubing through the outlet port on the front panel.
3. Attach the inlet and outlet tubing to the rest of the system with the PEEK fittings. Do not overtighten because the tubing is made from Teflon and is easily crushed.
4. Replace the side cover.

Loading the Control Software and Installing the ProStar 330 GPIB Card in the Dell Computer

The National Instruments control software must be loaded onto the Dell PC before the GPIB board is installed.

1. Insert the National Instruments CD into the CD-ROM drive.

   The standard Windows installation screen appears, followed by a screen called "Welcome to National Instruments GPIB Setup."
2. Choose **`Other`** and click on the **`Next`** button.
3. Accept the license and click **`Next`** on the line registrations screen.
4. **Start** the installation and **select all** of the components in the `Select the Components` window. Click on **`Next`**.
5. Click **`Next`** on the Install screen. After the installation is finished, click **`Finish`**.
6. Remove the National Instruments CD from the drive and shut down the computer.
7. After the computer has shut down, turn off the power and remove the power cord.
8. Remove the computer cover and install the GPIB board in one of the PCI slots, as shown in the *National Instrument Getting Started* manual.
9. After the board is installed, replace the covers, connect the power, and turn the PC on.
10. Windows should briefly display a `New Hardware Found` dialog box. If it does not, refer to the National Instruments manual for a way to manually force windows to find the GPIB board.
11. Attach the GPIB cable to the PCI GPIB board and then to the GPIB connector on the back of the ProStar 330. Do not connect this GPIB cable to any other ProStar module.
12. Set the **GPIB address** on the rear of the ProStar 330 to **9**.

Loading Polyview Software

1. Insert the Star 5.3 CD.
   - If the STAR installation wizard pops, up select **Install** and the select **Polyview 2000**.

- If the wizard does not pop up, use Windows Explorer to find the **`setup.exe`** file in the Polyview folders on the STAR 5.3 CD.

2. Click on **`Next`** on the welcome screen.
3. Enter name and company information in the user information screen. For the password, use the password found on the yellow sheet in the Polyview Communications kit.

Note: This password is different than the STAR 5.3 password.

4. Click **Next** on the software license agreement window.
5. Click **Next** on the information window.
6. Another window appears, which offers the option of selecting a file folder for Polyview 2000. Use the default setting.
7. A final window appears after the software is loaded saying the setup is complete. Click on **Finish**.

Turning the Lamp On and Off

1. Before turning the lamp on right after an installation, the LC system must be set up in the instrument configuration window of Star 5.3. Refer to the next chapter for instructions.
2. Once the instrument is configured, the lamp is turned on from Star 5.3 software by clicking the lamp on button on the ProStar 330 panel in System Control in Star 5.3. If the PC has recently restarted, the ProStar 330 software must be downloaded before it is possible to turn on the lamp.
3. The lamp on the ProStar 330 should be shut off from STAR software. Turning off the ProStar 330 by using the switch on the front panel requires the downloading of the software control code when the ProStar 330 is turned on again. This takes several minutes.

2.3 Installing the Stop-Flow Valve and Plumbing

The procedure in this section describes how to install the stop-flow valve and the plumbing. If you have an Analyte Collector with your system, skip to “HPLC Hardware Setup” on page 19—the Analyte Collector replaces the stop-flow valve.

For this procedure, use the 0.010-inch I.D. (blue striped) tubing to make the connections. Use the tubing cutter (provided) to cut the tubing to the desired lengths. A 12-foot length of red PEEK tubing will be used as the transfer line between the #4 port on the Stop-Flow valve and the IN port on the probe. This tubing can be shortened to reduce the delay time.

The 9012 LC pump ships with stainless steel tubing. You may use the stainless steel tubing instead of the PEEK tubing for connections between the LC pump, mixer, and purge valve. All connections between the 310 LC detector, column, and probe must use the PEEK tubing.

Unpack and Connect Plumbing

Unpack the stop-flow valve and place it on the table or cart near the injector and purge valve.

1. Connect a tube from the bottom port on the front of the pump to the large-volume mixer (Part No. 03-919622-01).
2. Connect a tube from the other end of the mixer to the #2 port on the stop-flow valve.
3. Connect a tube from the #5 port on the stop-flow valve to the top port on the front of the detector.
4. Connect a tube from the #3 port on the stop-flow valve to one of the high-pressure ports on the purge valve. Use the SSI ferrules provided in the kit to connect the tube to the high pressure port.

Prepare the Transfer Tube

Prepare the transfer tube, which is used to transfer solvents from the LC pump to the probe,.

1. Find the 12-foot red PEEK tubing from the installation kit.
2. Connect one end of the transfer tube to the #4 port on the stop-flow valve (Figure 8).

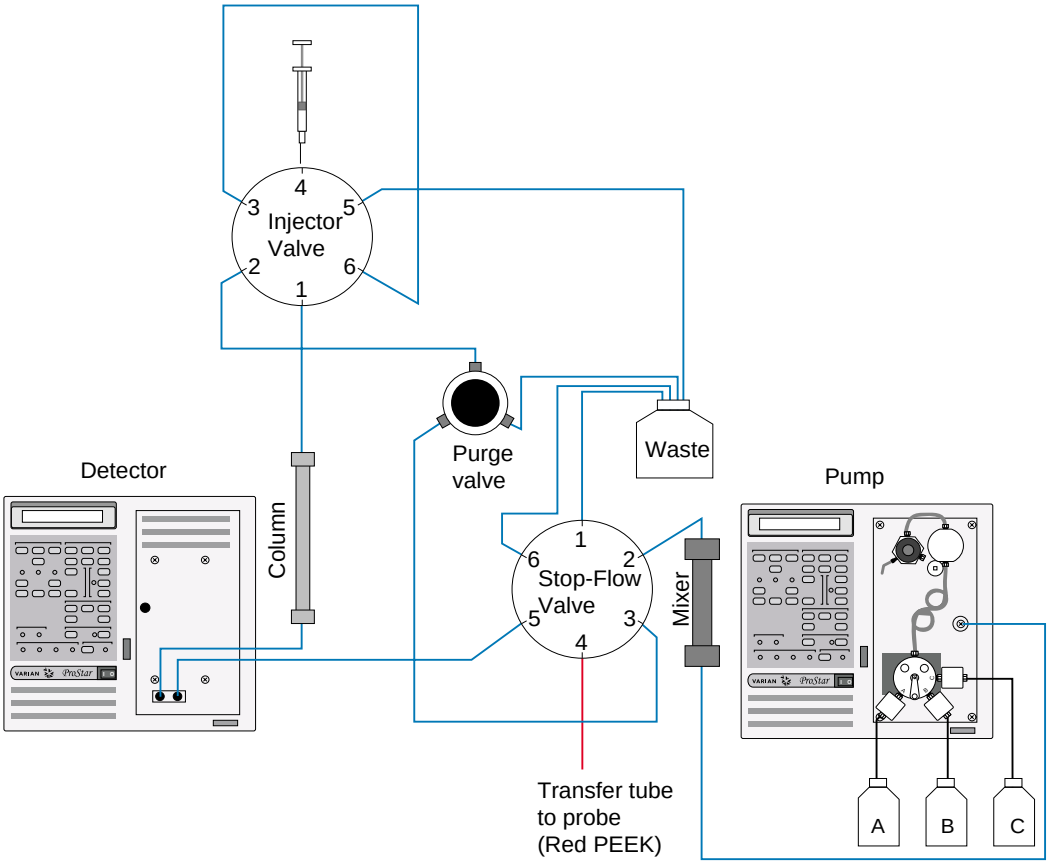


Figure 8. Stop-Flow Valve Plumbing Connection Diagram

3. For probe tests, this end of the transfer tube is temporarily connected to the pump outlet port.

4. If the Microflow probe is installed, connect the other end of the transfer tube to the LC connector on the probe labeled IN. Otherwise, place this end in a waste container near the magnet.
5. Connect tube from the #1 and #6 ports on the stop-flow valve to the waste container.

The stop-flow valve is now plumbed into the Varian HPLC system. Go to the next section to set up the PC workstation.

The table on the right gives an alternative plumbing configuration that maintains pressure during stop-flow.

Valve Port	*Connection*
4	plug
3	from pump mixer
2	to purge valve
6	to NMR
1	from detector
5	to waste

2.4 Unpacking and Assembling the LC STAR Workstation

Use the instructions provided in the *Varian Chromatography Systems* manuals to unpack and assemble the LC STAR workstation and to connect the GPIB (IEEE interface) cables to the pump and detector. After the LC STAR workstation is assembled, use the following procedures to install the ADC board in the PC, connect the PC cables, and install the LC Stop Flow Monitor software.

CAUTION: **Avoid equipment damage or data loss by keeping the LC STAR workstation PC well away from the magnet. Generally, keep the PC beyond the 5-gauss perimeter of the magnet. Refer to the *Installation Planning Guide* for magnet field plots.**

This procedure describes how to install the ADC board into the LC STAR workstation. The ADC board in the PC provides the basic analog and digital I/O interface between the LC STAR workstation, LC hardware, and the NMR system.

1. Make sure the PC power is off, but leave the power cord connected. Follow anti-static procedures because some of the parts in the PC and on the boards are static sensitive.
2. Remove the cover from the PC.

   The PC contains a number of ISA-type expansion slots. One of the expansion slots should already contain the GPIB IEEE interface board. The ADC board can be installed in any one of the other free expansion slots.
3. Choose the expansion slot for the ADC board. If the expansion slot has a slot cover on the back panel of the PC, remove it.
4. Locate the ADC board in the kit and carefully remove board from its packaging. Be sure to hold the board only by its edges.
5. Install the ADC board in the empty slot.

   Carefully align the board connector with the connector inside the PC—the rear panel connector on the board must face the rear of the PC. Then, using your thumbs, gently push the board into the connector until the board is fully seated.

   When correctly seated, the 50-pin ribbon cable connector on the ADC board should be accessible through the back of the PC.
6. Replace the cover on the PC.

   The ADC board is now installed.

2.5 Connecting the Stop-Flow Cable

This procedure describes how to connect the stop-flow cable between the PC, NMR console, pump, stop-flow valve, and the detector. The stop-flow cable begins at the interface box at the 25-pin D-shell connector. The harness then divides into cables that connect the LC with the NMR console and cables that connect to the LC hardware. The PC connects to the interface box with a ribbon cable from the ADC board.

- Figure 9 shows a diagram for connecting the stop-flow cable to the 310 detector.

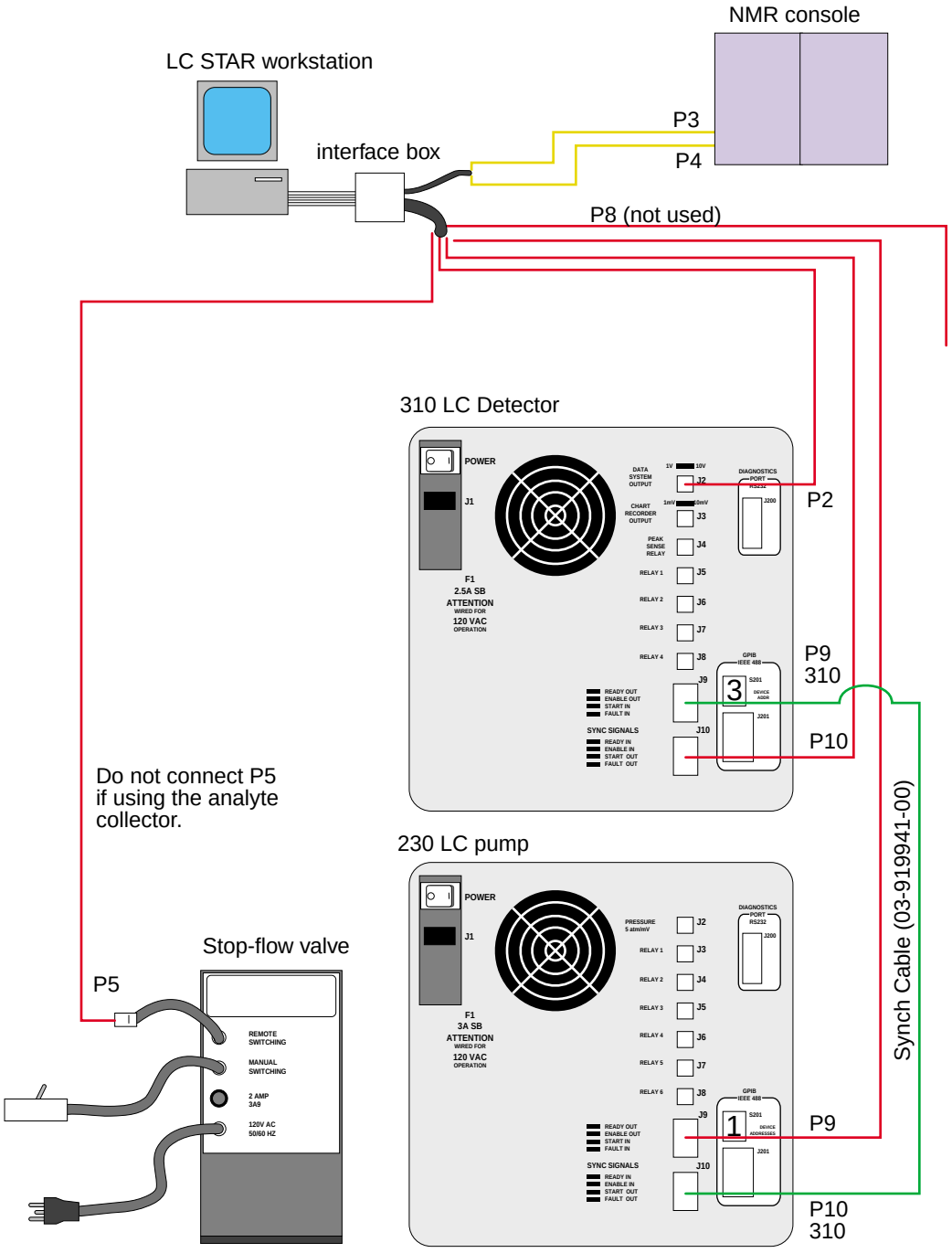


Figure 9. Diagram for Connecting the Stop-Flow Cable with 310 Detector

- Figure 10 shows a diagram for connecting the stop-flow cable to the 330 detector.

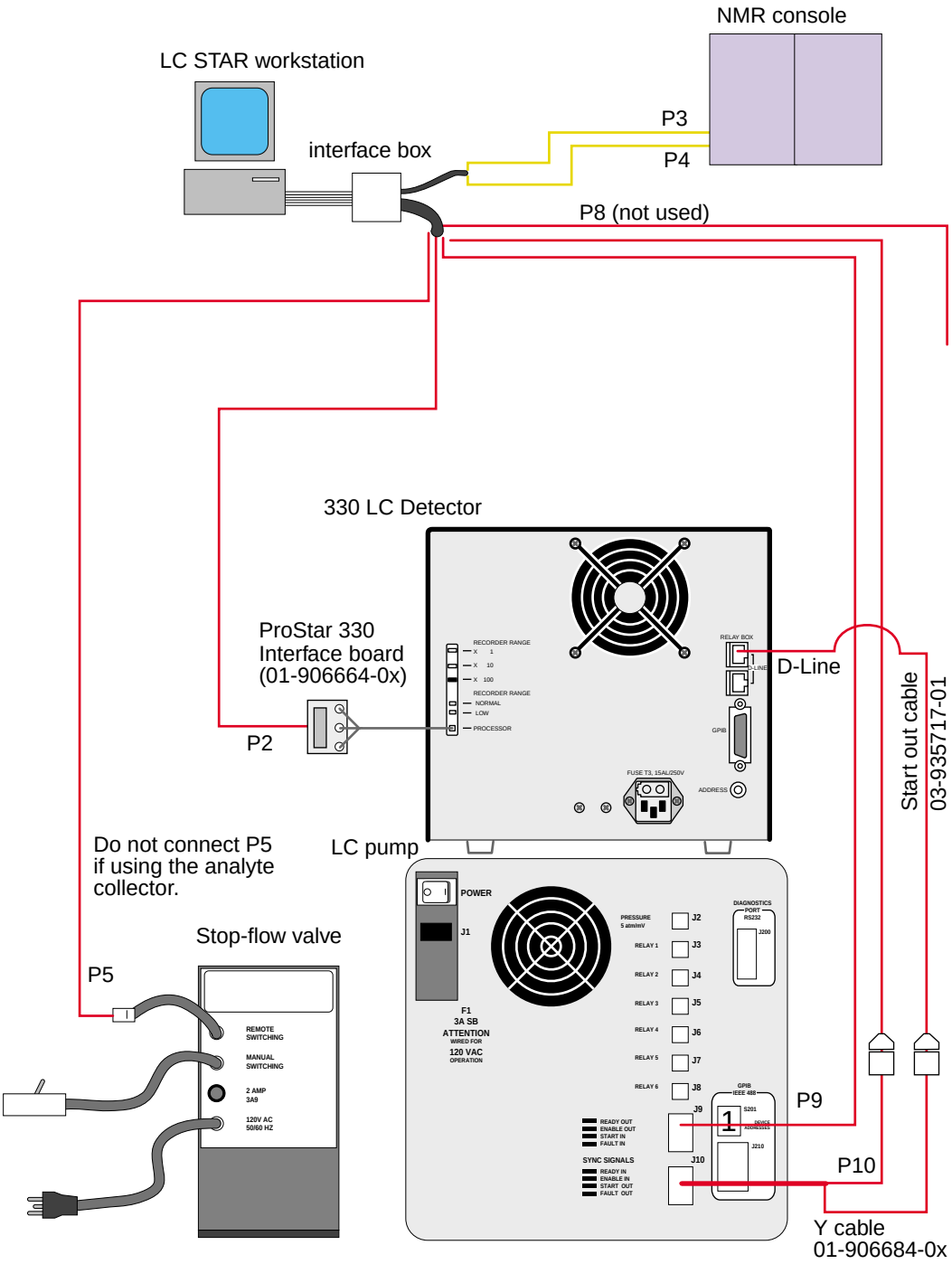


Figure 10. Diagram for Connecting the Stop-Flow Cable with 330 Detector

1. Set the jumpers in the interface box (see Figure 11) for either UNITY*INOVA*/ UNITY*plus* or UNITY as follows:

   a. Loosen the four screws on the side of the interface box.

   b. Remove the interface box cover.

   c. Set jumpers J2 and J3 for UNITY*INOVA*/ UNITY*plus* or UNITY:

   UNITY*INOVA* or UNITY*plus* – set J2 and J3 to the side nearest the U+/I label.

   UNITY – set J2 and J3 to the side nearest the UNITY label

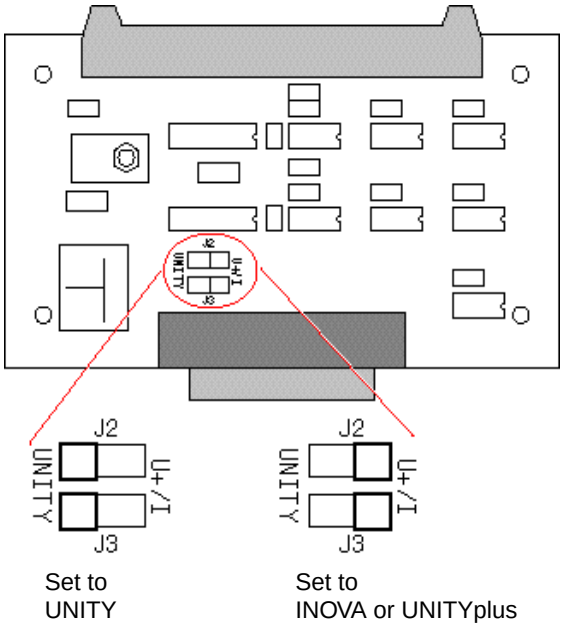


Figure 11. Interface Box Jumper Settings

   d. Replace the cover and tighten the four screws.

2. Connect the ribbon cable between the ADC board in the PC and the interface box.

3. Connect the two cables to the NMR console as follows:

   - UNITY*INOVA*—Connect one cable (P3) to SPARE 1 on the Breakout panel. Connect the other cable (P4) to EXT TIME BASE on the DAC board. Figure 12 shows the connectors on the Breakout panel.

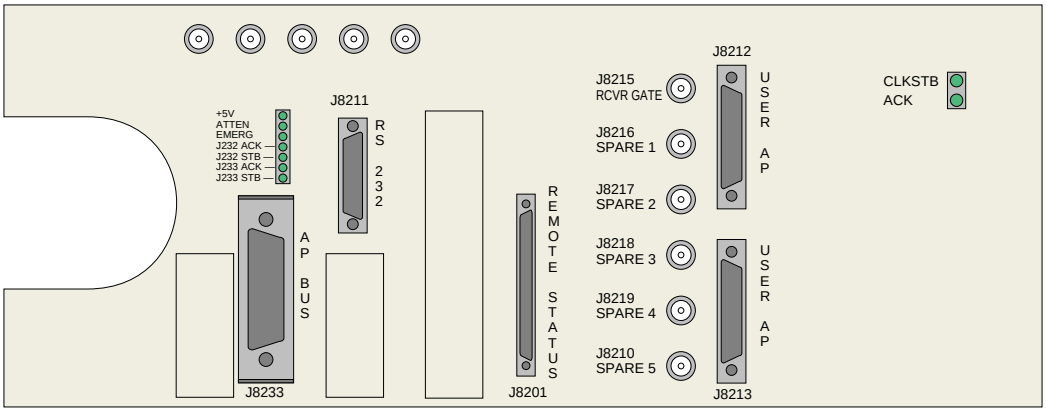


Figure 12. UNITY*INOVA* Breakout Panel Connectors

- UNITY*plus*—Connect one cable (P3) to SPARE 1 on the Pulse Sequence Controller board (Figure 13). Connect the other cable (P4) to Ext Time Base on the Pulse Sequence Controller board.

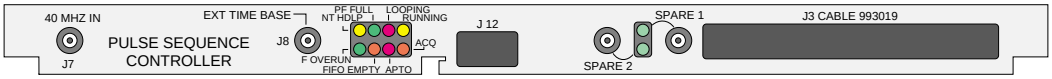


Figure 13. UNITY*plus* Pulse Sequence Controller Board, Front Panel

- UNITY—Connect one cable (P3) to SPARE 1 on the XL Interface board (in the 12-pin jumper pack J5, connect one wire to pin 7 and one wire to ground). Connect the other cable (P4) to Ext Time Base on the Acquisition Controller board.

4. Refer to Figure 9 for the 310 or Figure 10 for the 330. Then make the following connections:

   a. Connect the cable labeled P2 to J2 (DATA SYSTEM OUTPUT) on the detector.

   b. Connect the following cables according to the type of detector:

      310 detector – connect the cable labeled P9 to J9 on the LC pump. Connect the Sync cable (03-919941-00) between J9 on the 310 detector and J10 on the LC pump. Connect the end labeled P9 9010 to J9 on the 310 detector, and connect the end labeled P10 310 to J10 on the LC pump.

      330 detector – connect the cable labeled P10 to J10 on the LC pump. Connect the cable labeled P9 to the J9 on the LC pump.

   c. If an analyte collector *is not* used, connect the cable labeled P5 to the connector on the REMOTE SWITCHING cable of the stop-flow valve. If an analyte collector *is* used, do not connect P5.

5. Make sure the thumbwheel switch (S201 DEVICE ADDRESS) on the back of the LC pump is set to 1; if not, set it to 1.

6. Make sure the thumbwheel switch (S201 DEVICE ADDRESS) on the back of the LC detector is set to 3; if not, set it to 3.

The stop-flow cable is now connected.

2.6 Installing the Powered Event Module

This procedure describes how to install the air line used by the Powered Event Module (PEM), shown in Figure 14. This procedure also describes how to connect the PEM to the other LC hardware.

The two procedures in this section describe how to connect the data cables and install the air line into the source filter/regulator air line. The PEM requires a clean air/nitrogen source of 60 psi minimum pressure.

Connecting the Injector Valve and the Pump

This procedure describes how to install the air lines to the injection valve and how to connect the data cable between the Powered Event Module and the Detector. Leave the power cord disconnected and the power switch off until all other connections are made.

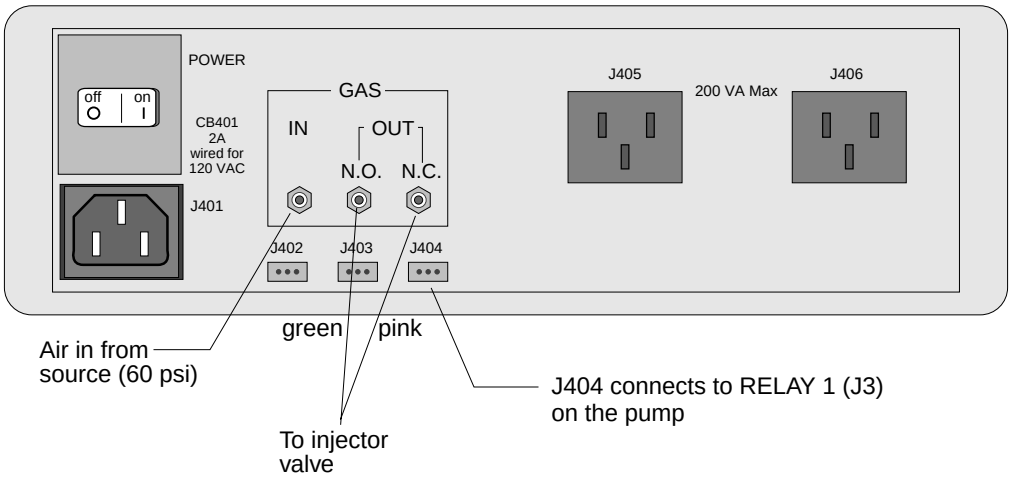


Figure 14. Powered Event Module, Back Panel Connectors

1. Connect a cable from J404 on the back of the PEM to the connector on the back of the LC pump labeled RELAY 1 or J3.
2. Connect one end of the long source air tube (1/8-inch O.D.) to the hose barb labeled IN (see Figure 14). Leave the other end of this tube disconnected until the next procedure.
3. Make the air connections between the PEM to the injector valve as follows:
   a. Locate the two hose barbs on the side of the injector valve (see Figure 15).
   b. Connect one end of the pink 1/8-inch O.D. tube to the hose barb labeled N.C. Connect the other end of this tube to the hose barb on the injector valve that is farthest from the mounting plate (see Figure 15).

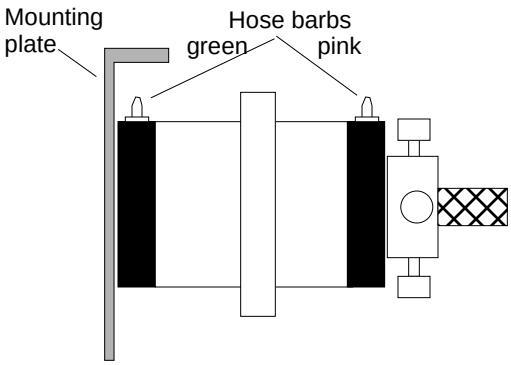


Figure 15. Injector Valve, Top View

   c. Connect one end of the green 1/8-inch O.D. tube to the hose barb labeled N.O. Connect the other end of this tube to the hose barb on the injector valve that is closest to the mounting plate (see Figure 15).
4. Go to the next procedure to connect the source air.

Connecting the Source Air

This procedure describes how to connect the regulator assembly (00-990668-00) to the filter/regulator assembly used by the spectrometer. The new regulator is used to regulate the air used by the Powered Event Module (PEM) and attaches to the cross connector as shown in Figure 16. You will need a 1/4-inch Allen wrench. Wrap all threads with Teflon tape before assembly.

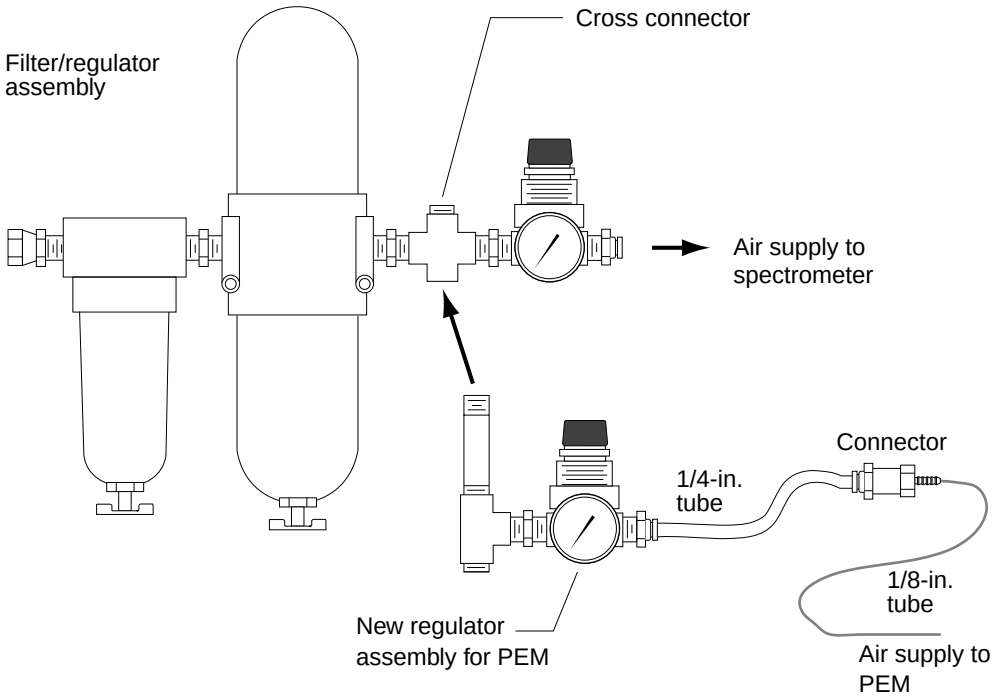


Figure 16. Connecting the Regulator to the Cross Connector

1. Make sure no sample is in the probe or magnet.
2. Locate the filter/regulator assembly that supplies the system on which you are installing the LC-NMR.
3. Turn off the air flow to the filter/regulator assembly.
4. Use a 1/4-inch Allen wrench to remove the plug from the bottom (or top) of the cross connector.
5. Screw the new regulator assembly into the cross connector as shown in Figure 16.
6. Connect one end of the 1/8-inch PEM supply tube to the 1/8-inch hose adaptor (28-849916-00). Use a connector (28-859986-00) to connect the hose barb to the 1/4-inch tubing connected to the regulator.
7. Connect the other end of the 1/8-inch PEM supply tube to the IN connector on the back of the PEM. You might need the connectors 28-849916-00 and 28-859986-00.
8. Turn on the air flow (60 psi) to the filter/regulator assembly until the source air pressure is 60 psi.
9. Turn the knob on the regulator for the Powered Event Module until the pressure reads 60 psi.

   The air line for the Powered Event Module is now installed.

2.7 Leak-Testing the System

This procedure describes how to perform a leak test of the after the stop-flow valve and plumbing is installed. A system leak test is available through the Extended Diagnostics of the 9012 LC pump. Two leak test modes are provided: constant pressure or pressure decay.

- In the constant pressure mode, the pump operates to maintain a set pressure. The reported leak rate (in microliters per minute) is the flow rate required to maintain the set pressure. You should run the constant pressure mode leak test first.
- In the pressure decay mode, the pump pressurizes the hydraulics to the set pressure and then stops. The decay of the pressure is then monitored as a function of time. The pressure decay mode is useful for further diagnostics if the system fails the constant pressure mode test.

Either leak test can be performed on the entire high-pressure flow path (*not the probe*). When testing the entire system, the plug must be placed upstream from the detector because most detector flow cells are not designed to operate at high pressure.

WARNING: **Hazardous chemicals are under pressure. Use proper eye and skin protection.**

CAUTION: **Do not leak test the probe or detector cell. The high pressures used for the leak test will damage the flow cells in the probe and detector. Never exceed 2.0 mL/min flow rate through the probe.**

Running the Leak Test

The following steps describe how to run the leak test in either constant pressure or pressure decay mode.

1. Carefully inspect the system for leaks (drips or salt crystals). Any obvious leaks must be corrected before continuing. Locations to check include the piston seal drain, the prime/purge valve, and all nuts and ferrules.
2. Connect the solvent to be used for the leak test to the A proportioning valve. Prime the pump and purge solvent through the entire system to remove any air (These procedures are described later in “Using the LC Pump” on page 36). Choose a solvent compatible with the last solvent used in the HPLC system.
3. Remove the fitting from the outlet side of the column. Replace it with a known plug.

CAUTION: **The hydraulic path must be plugged in such a way that the pressure transducer is always included in the closed system. Operation of the leak test without the pressure transducer in line could result in damage to the pump.**

4. On the 9012 LC pump, press and hold the SELECT key while simultaneously turning on the power.

   This will display the Extended Diagnostics Menu.
5. Scroll to the Leak Test using the NEXT LINE key. Press START.
6. Select either Constant Pressure or Pressure Decay mode using the INCREASE or DECREASE key. Press NEXT LINE.

7. Set the pressure for the leak test. The pressure may be set from 10 to 410 atm. A good test pressure would be slightly higher than the expected operating pressure. Typically 200 atm is used. Press NEXT LINE.
8. Close the prime valve if it is open. Press START.

   After the pressure has stabilized, determine if a leak exists. If so, look for visible signs of solvent leaking at one of the fittings.

Note: *If a leak is found, do not tighten fittings while the system is pressurized and do not overtighten the fitting.* Instead do the following:

   a. Press STOP to stop the leak test.
   b. Slowly release the pressure by carefully opening the prime valve.
   c. After the pressure has dropped to zero, tighten the fitting just enough to correct the leak.
   d. Repeat the leak test.

9. When the leak test is finished, press STOP. Slowly release the pressure by carefully opening the prime valve.

   After the pressure has dropped to zero, remove the plug and reconnect the solvent line. Turn off the power switch and then turn it back on to restore normal operation of the 9012 LC pump.

Interpreting Leak Test Results

The constant pressure leak test displays the leak rate directly in µL/min. Because of the extreme sensitivity of this test, take care in interpreting the reported leak rate. Relate the leak rate to the operations flow rate to determine the significance of a reported value. For example, a leak rate of less than 1 µL/min will be unimportant at a flow rate of 1mL/min.

The pressure decay mode displays a pressure drop and an elapsed time. If the hydraulics being tested include the standard damper, the leak rate in µL/min may be estimated by multiplying the pressure drop (in atm) by 1µL/atm and dividing by the elapsed time.

Both test modes are sensitive to fluctuations in ambient temperature, which can lead to thermal expansion or contraction of the liquid in a closed system. This can cause small fluctuations in the pressure or leak rate readings, but if a leak exists, it should become apparent after the system has been under pressure for 5 or 10 minutes. As a rule of thumb, a drop of water is approximately 50 µL. For organic solvents or mixtures of organic and water, a drop is typically smaller than 50 µL. Therefore, with a leak rate exceeding 25 µL/min, liquid should be visible at one of the fittings after several minutes of test. Lower leak rates with a high percentage of organic solvent may not allow a visible amount of solvent to accumulate because of evaporation. If no visible leak is seen, then the pump head may be leaking internally (piston seal or inlet valve) or, if included in the test, the injector valve might be leaking internally.

If the constant pressure leak test produces a higher leak rate than is tolerable after running it for 10 minutes and no visible leaks can be found, run the pressure decay leak test. If the results of this test are acceptable, then the fittings between the outlet check valve and the plug are leak tight and an internal leak in the pump head is indicated. Check carefully around the drain passage at the bottom of the pump head. If no leakage appears here, then the inlet valve may be leaking, since leaks here can not be seen externally.

If the results of the tests are not conclusive, the leak tests should be repeated before changing any of the hardware in the pump head.

2.8 Using the LC Pump

This section describes how to use the 9012 Solvent Delivery System (LC pump) from the front panel keypad for the following operations:

- Creating an Initial Pump Method
- Creating a Relay Program
- Priming the Pump
- Flushing the Probe Flow Cell
- Purging the Transfer Tube
- Transferring a Solvent into the Probe

You will use these procedures when you are installing and testing the probe. All pump operations described here are selected on the front panel of the pump. Refer to the *Varian Chromatography Systems* manual for further details on using the pump.

To use the STAR Chromatography software for method building, see the LC-NMR operation chapter in the manual *User Guide: Liquids NMR*.

Creating an Initial Pump Method

This procedure describes how to create a method for testing the probe and calibrating the system. The method created here uses parameters that are optimized for probe testing and system calibration. You can create other, more intricate methods using the documentation provided by Chromatography Systems or the LC-NMR operation chapter in the manual *User Guide: Liquids NMR*.

A *method* is a group of parameters that control the pump. You use the front panel keypad on the pump to create this method.

1. If not already done, turn on the pump power as described in the Chromatography Systems manual.
2. Press the **STATUS** button in the OPERATION menu.

   The display on the front of the pump shows a read-only status of current pump conditions. Figure 17 shows the typical initial status display for method 1.

Meth	%A	%B	%C	Flow	Pres	Relays	Time
1	100	0	0	1,00	0	------	0.00

Figure 17. Status Display for Method 1

3. Press the **METHOD ACTIONS** button in the TABLE SELECT menu. The display shown in Figure 18 appears. The cursor should be under `METHOD SELECT`. If not, use the cursor buttons to move the cursor.

Method	Error	Method	Metho	Run	Table
Select	Log	Copy	Delet	Log	Print

Figure 18. Method Actions Display

4. With the cursor under `METHOD SELECT`, press SELECT.

The display shows `Select Method – Press Select` and allows you to choose a method number. We will be setting up method 1, so press the INCREASE or DECREASE button until `1` is displayed. Then press SELECT.

The display shows `Method 1 Selected`.

5. Press **SET CONDITIONS**. The display shown in Figure 19 appears.

Method	Endtime	Action	Pmin/Pmax	Equil Time
1	6.00	Equil	0 / 300	0.00

Figure 19. Method 1 Conditions Display

6. Make the values the same as shown in Table 1.

   Use the **CURSOR**, **INCREASE**, and **DECREASE** buttons to change the values.

7. After the values are set to those shown in Table 1, press **STATUS** to return to the status display. Then go to the next procedure to create a relay program.

Table 1. Pump Method 1 Conditions

Field	*Value*
Endtime	6.00
Action	Equil
Pmin	0
Pmax	300
Equil Time	0.00

Creating a Relay Program

This procedure describes how to create the initial relay program for testing the probe and calibrating the system. If you have other devices connected to your pump, such as an autosampler or analyte collector, you might need to create a more detailed relay program, as described in the Chromatography Systems manuals. For testing the probe and calibrating the system, you only need to create a program for relay 1, which controls the injector valve.

Relays can be time programmed to control six external event relays. You will use the front panel keypad on the pump to create this relay program.

1. Press the **RELAYS** button in the TABLE SELECT menu.

   The relay display for method 1 appears as shown in Table 20. The dashes (–) under `Relays On` represent the six external event relays on the back of the pump.

Method	Line	Time	Relays On
1	1	0.00	------

Figure 20. Relay Program Display

2. Press **NEXT LINE** in the EDIT menu. If the system says that no line exists, press **INSERT LINE** to add a new line.

   Line 2 is created. Notice that the time has changed from 0.00 to 0.01.

3. Use the **CURSOR** buttons to move the cursor under the first dash under `Relays On`. This represents relay 1, which is connected to the injector valve. Press **INCREASE** to turn on relay 1.

4. Press **NEXT LINE** in the EDIT menu. If the system says that no line exists, press **INSERT LINE** to add a new line.

Line 3 is created.

5. Move the cursor to the **Time field** and push the **INCREASE** button until the time is 1.00.
6. Move the cursor to the first relay under the **Relays On field** and push **INCREASE** to turn off relay 1.

   The relay program for method 1 is finished. Table 2 shows three lines of the relay program.

Table 2. Initial Relay Program for Pump Method 1

Method	*Line*	*Time*	*Relays On*
1	1	0.00	– – – – – –
1	2	0.01	1 – – – – –
1	3	1.00	– – – – – –

Priming the Pump

This procedure describes how to prime the pump after new solvents are attached to the inlet ports. The procedure is for a fully installed LC-NMR system.

CAUTION: **To avoid unnecessarily high fluid pressures in the NMR probe, never exceed 2.0 mL/min flow rate through the probe. The preferred flow rate is 1.0 mL/min or less. This requires extra attention during pump priming and purging procedures. For the same reason, the maximum length of output tubing from the probe to the waste receptacle is one meter, and do not use output tubing that is less than 0.010 in. inside diameter. Beware of clogged or crimped output tubing.**

1. Disconnect the blue tube from the bottom port on the LC pump. Figure 21 shows this port.
2. Disconnect the red tube from the #4 port on the stop-flow valve and connect it to the LC pump output port (the bottom port on the LC pump, shown in Figure 21).
3. **Disconnect the transfer tube from the probe** and place the open end of the transfer tube into a waste container near the probe.
4. Connect the new solvent bottles to the pump inlets using dry pieces of 1/8-inch tubing. Inlet filters are not recommended for LC-NMR. Make sure each bottle is labeled and dated.
5. Open the priming valve on the front panel of the pump (see Figure 21).

   Turn the prime valve about one turn counterclockwise. Place a waste container under the drain line during the priming process.
6. Press **A** to prime the inlet port A.

   The pump starts, the PRIME LED lights, and the solution connected to inlet port A begins to flow through the prime valve into the waste container.

   When the liquid flow from the prime drain is continuous and free of bubbles, press STOP.
7. Repeat step 4 for inlet ports B and C.
8. After priming is finished, close the prime valve by turning it clockwise.
9. Reconnect the tubes that you disconnected in steps 1 and 2:
   a. Disconnect the red tube from the bottom port on the LC pump and connect it to the #4 port on the stop-flow valve.
   b. Reconnect the blue tube to the bottom of the LC pump.

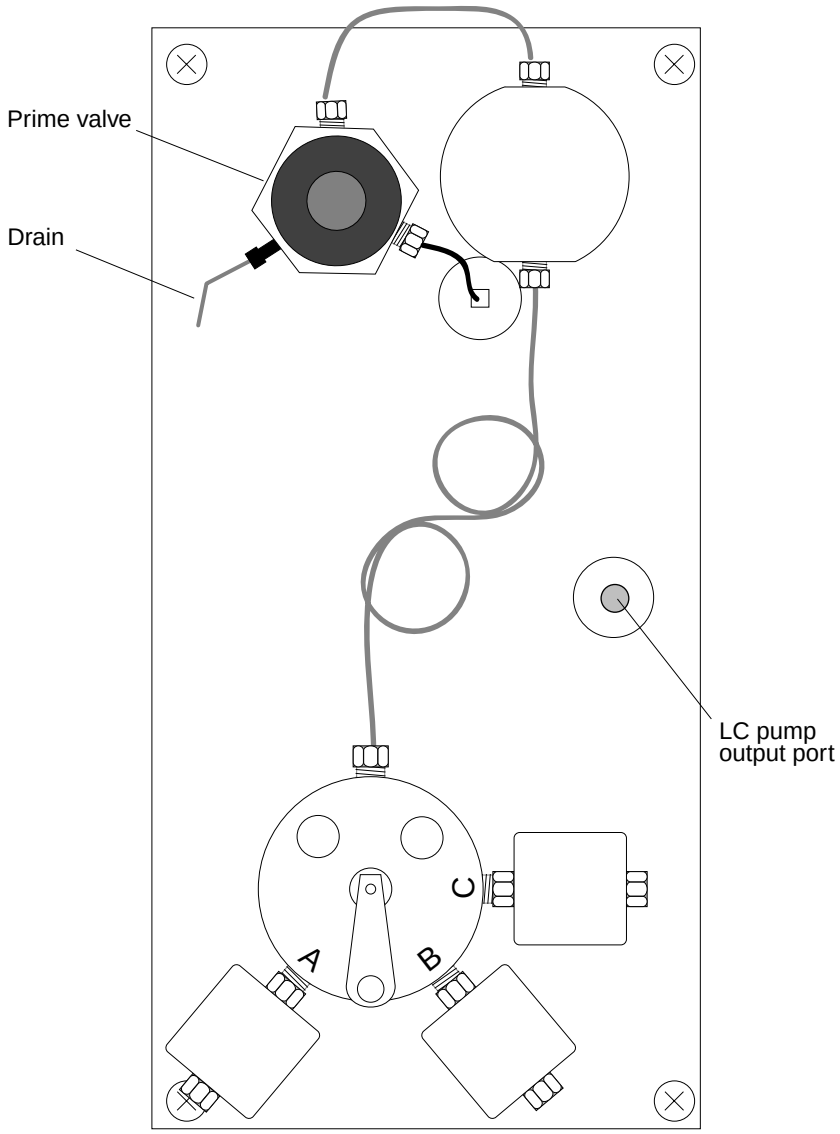


Figure 21. Prime Valve and Drain on Front of LC Pump

The pump is now ready to transfer solvents to the probe.

Flushing the Probe Flow Cell

This procedure describes flushing out the probe flow cell. To prevent contamination, the probe flow cell must be flushed out with D_2O after you finish using a particular solvent. The procedure is for a fully installed LC-NMR system.

1. Connect the transfer line to the probe.
2. Use the **CURSOR** buttons to move the cursor under **%A**, or whatever inlet port is attached to the bottle containing the D_2O.
3. Press the **INCREASE** button until the value is **100** under **%A** (or whatever inlet port is attached to the D_2O flushing solution).

4. Use the **CURSOR** buttons to move the cursor under **Flow**.
5. Use the **INCREASE** or **DECREASE** buttons to set the value under **Flow** to **1.00**. This sets the pump rate at 1.0 mL/min.
6. Press the **RESET** button twice.
7. Press **PUMP**.
8. Pump the flushing solution through the probe for at least 3 minutes.
9. Examine the output of the probe for steady flow into the waste container.
10. The flow characteristics will be either steady-flow or drop-wise depending on the solvent.
11. After 3 minutes, press **STOP** to stop the pump.

Purging the Transfer Tube

This procedure describes purging the transfer tube of old solvent and filling it with new solvent. The procedure is for a fully installed LC-NMR system.

CAUTION: **To avoid unnecessarily high fluid pressures in the NMR probe, never exceed 2.0 mL/min flow rate through the probe. The preferred flow rate is 1.0 mL/min or less. This requires extra attention during pump priming and purging procedures. Beware of clogged or crimped output tubing.**

Note: Before starting, make sure the probe is disconnected from transfer line.

1. Select **100% A, B or C** for the specific inlet port desired. Select a flow rate of 1.0 mL/min and press the **RESET** key twice.
2. Make sure the prime valve is open; then, press the **PRIME** key and prime the transfer line for about one minute.
3. Press **STOP**.

   Purging pumps the solvent at 10 mL/minute at concentrations specified by the %ABC FLOW proportions. The purge operation ignores the specified flow rate and automatically pumps at 10 mL/minute.

Transferring a Solvent into the Probe

This procedure describes how to use the pump to transfer a solvent into the probe flow cell. The procedure is for a fully installed LC-NMR system.

1. If the transfer tube is not connected to the probe, connect it to the IN port on the probe body.
2. Press **%ABC FLOW**.

   The value 100 should already appear under whatever inlet port is attached to the solvent you want to transfer. If not, use the CURSOR buttons and the INCREASE or DECREASE button to set the value to 100.

CAUTION: **To avoid unnecessarily high fluid pressures in the NMR probe, never exceed 2.0 mL/min flow rate through the probe. The preferred flow rate is 1.0 mL/min or less. This requires extra attention during pump priming and purging procedures. For the same reason, the maximum**

length of output tubing from the probe to the waste receptacle is one meter, and do not use output tubing that is less than 0.010 inches inside diameter. Beware of clogged or crimped output tubing.

3. Use the **CURSOR** buttons to move the cursor under **`Flow`**.
4. Use the **INCREASE** or **DECREASE** buttons to set the value under **`Flow`** to **1.00**. This sets the pump rate at 1.0 mL/min.
5. Press the **RESET** button twice.
6. Press **PUMP**.
7. After **5 minutes**, press **STOP**.

   The solvent is transferred into the probe flow cell and the probe is ready for NMR.

Chapter 3. Software Installation: Cascade, Drivers, STAR 5.3

Sections in this chapter:

- 3.1 “Loading the VEE Samples” this page
- 3.2 “Installing Software Drivers” this page
- 3.3 “Installing Cascade” page 44
- 3.4 “Resolving Sound Card Conflict on DELL Computers” page 45
- 3.5 “Reinstalling Cascade for Windows 95/98” page 45
- 3.6 “Setting Up STAR 5.31” page 46
- 3.7 “Setting Up the Ethernet Connection” page 50

3.1 Loading the VEE Samples

1. Insert the VEE Samples disk in the A: drive.
2. Use Windows Explorer to look at the contents of the disk.
3. Double-click on the setup icon.
4. Follow the instructions.

Note: If the VEE Samples disk is missing from the shipment, make a copy of the VEE samples from the LC-NMR training course disk.

3.2 Installing Software Drivers

Driver Installation

The DT2801 driver must be installed so that system can recognize the Data Translations ADC board.

1. Insert the floppy disk labeled *DT2801 Device Driver for win3.11 and win95*.
2. Go to the **Control Panel** and select **Add New Hardware**.
3. Select **Next**.
4. Answer **No** to “Do you want windows to search” and click **Next**.
5. Select **Sound, Video and Game Controllers for hardware type** and click **Next**.
6. Click the **Have Disk** button and browse to the **A: drive**. Go to the **win95** directory.

7. Select **dt280~1.inf** and click **OK**.

   The window should now show **A:/wind95**.

8. Click **OK**.
9. Select **DT-Open Layers DT2801 Series** and click **OK**.
10. Click **Finish**.
11. Double-click on the **Multimedia** icon in the **Control Panel**.
12. Select the **Advanced** tab.
13. Double-click **Media Control Devices**.
14. Double-click **DT-Open Layers DT2801 Series Device Driver**.
15. Click the **Settings** button.
16. If you do not see **DT2801**, select **ADD** and add the **dt2801** at hex address **0x2EC**.
17. Under General Configuration, change the DMA Channel number as follows:
    - For **DELL GX1** Systems, select **DMA Channel=3**.
    - For **other** systems, select **DMA Channel=1**.
18. Click the **Update** button. Click **Close**.

Driver Load Check

Reboot and check that the drivers are loaded.

1. Reboot the computer
2. After the Win 98 picture is displayed, the screen should go blank except for one line at the top of the screen that reads `Voltdad.386 Data Transactions Data Acquisition`. This indicates that the board and drivers have been loaded properly. This must be correct before loading Cascade

3.3 Installing Cascade

The Cascade program coordinates the actions of the HPLC pump and stop-flow valve with acquisition of data by the NMR system.

Installing the Cascade program on the LC STAR workstation running Microsoft Windows 98 involves loading and initializing the software.

Loading the Cascade Software

1. Insert disk 1 into the floppy drive.
2. Click on **Start**, select **Settings**, and open the **Control Panel**.
3. Select **Add/Remove Programs**.
4. Click on the **Install** button and follow the directions on the screen.

   Windows should start running `setup.exe` from the floppy.

5. Insert disk 2 when asked to do so.
6. **Restart** the computer.

You should see a message about `VTDAD.386` during the startup process.

Initializing Cascade

1. Click on **Start**, select **Programs**, and launch **Cascade**.

   After Cascade starts, a window pops up with the status of the A/D board.
2. Click **OK**.
3. Pull down the **Setup** menu and select **Communications**.
4. Set the **loop increment delay** to **50** and the **loop read delay** to **10,000**.
5. Change other parameters (such as run time) as necessary.
6. **Close** the Configuration window.

3.4 Resolving Sound Card Conflict on DELL Computers

A memory conflict between the onboard sound card in the DELL computer and the DT2801 Data Translation might exist. The conflict appears as a yellow exclamation point next to one of the drivers in the Control Panel System Manager. Fix the conflict as follows:

1. Double-click on the **System** icon in the **Control Panel**.
2. Click the **Device Manager** tab.
3. Select the **Crystal PnP Audio MPU-401 Compatible** entry.
4. Click the **Properties** button.
5. Click the **Resources** tab.
6. Remove the check by **Use automatic settings**.
7. Select **Input/Output Range**.
8. Click the **Change Settings** button.
9. Use the scroll arrows to find an address that does not use 0330-033F (e.g., 03E8-03E9).
10. click **OK** three times to finish.

3.5 Reinstalling Cascade for Windows 95/98

If it becomes necessary to reinstall Cascade, use the steps in this section.

1. **Restart** the computer.
2. Select **Add/Remove Programs** from **Control Panel**.
3. Select **Cascade** in the list of software. Click on **Add/Remove**.
4. Answer **Yes** to remove any shared files.
5. **Restart** the computer.

   Ignore the warning "`The file vdtdad.386 cannot be found.`"
6. Select **Add/Remove Programs** from **Control Panel**.
7. Choose **Install** and instert the first Cascade disk.

Windows starts installing Cascade. The message that Windows has detected a previous version of Cascade appears.

8. Remove the disk when the installation is finished and **restart** the computer.

Note: If Cascade is installed without drivers, there is a flag in the registry editor that sets `noboard` to `true` (The path is `HKEY_CURRENTUSER\Software\VB` and `VBA Program Settings\Cascade\Hardware`). This limits Cascade to a safe mode where all control buttons are inaccessible (grayed out). Subsequent installations of Cascade with the A/D board drivers present might set this flag to `false`, which means the flag must be set manually.

3.6 Setting Up STAR 5.31

This section describes how to set up STAR 5.31 software.

- "Loading STAR 5.31" this page
- "Building a Method in STAR 5.31" on page 48
- "Building a Method in STAR 5.31" on page 48

Loading STAR 5.31

1. Install the STAR 5.31 software from the CD-ROM.
   a. Insert the STAR 5.31 CD-ROM and click **INSTALL**.
   b. Select **STAR Chromatography Work Station**.
   c. Read the license agreements and click **Agree**.
   d. Enter **name**, **company**, and **serial number** (from bright yellow sheet).
   e. Install the software into the directory **`C:/STAR`**.
   f. In the LC Components window, select **Example Data** file and **Advanced Applications**. If you have a 9065 detector, click on **9065 driver box**. Click **NEXT**.
   g. Answer **No** to the question `Do you want STAR tool bar to start automatically?`
   h. Press RETURN and EXIT.

   After the software is loaded, a STAR icon appears in the left side of the screen.
2. Load STAR Update #2.
   a. Insert the first of the three floppy disks.
   b. Click on the **FLOPPY A** icon to open files.
   c. Double-click on the blue computer icon labeled **set up**.
   d. If necessary, enter **`A:\`** for the path.

      The software begins to load.
   e. At the prompt, eject the disk and insert the second disk. `A:\` is the correct path.
   f. At the prompt, eject the disk and insert the third disk. `A:\` is the correct path.
3. If using the ProStar 330, install Polyview 2000 from the CD-ROM. This requires a superuser password. Refer to "Loading Polyview Software" on page 24.

4. Disable modules that are not being used from the STAR toolbar.
5. Set up the STAR software.
    a. Click on the **STAR Tool bar** icon (left side of the screen).
    b. In the right side of the tool bar, at the top of the screen, click the right mouse button and select **Module Disable/Enable** from the menu that appears.

       An upper and lower box appears. All possible modules made by Varian are enabled in the top box.
    c. Highlight the module that you have *not* installed on your system by clicking on their names. After the modules are selected, click the **disable** button.

       The modules that you have disabled will appear in the lower box where you can enable them later if necessary. The modules that you have left enabled appear in the upper box.
    d. Click **OK** when you are finished.
6. Set up the LC instrument from the System Control icon (upper left icon on the STAR tool bar). Drag the icons for the pump and detector into instrument 1 as shown in Figure 22. Double click on one of them when the icon outline turns black. An image of the front panel of the module should then be displayed, similar to Figure 23.

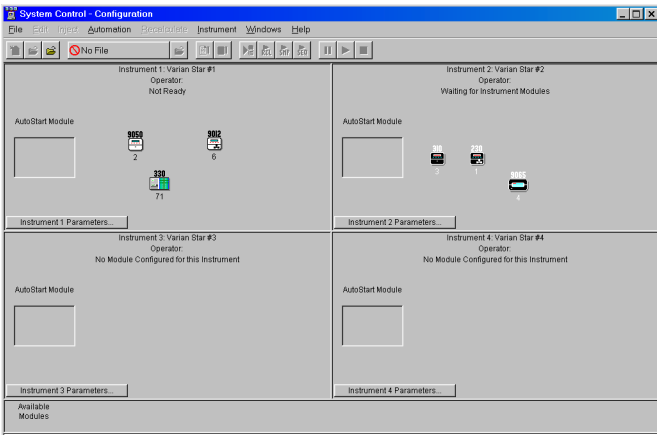


Figure 22. STAR Configuration Window

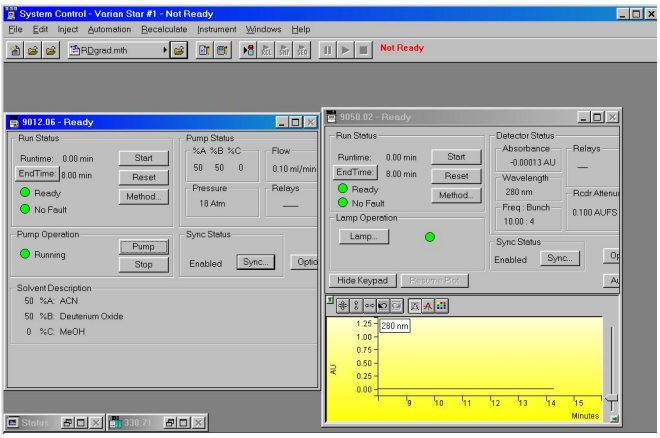


Figure 23. STAR System Control Window

Building a Method in STAR 5.31

This procedure describes building a method in STAR 5.31.

1. Enlarge the pump and detector windows to monitor the pump and detector states.
2. Click on the **method builder icon** (second from the left) in the STAR toolbar, similar to Figure 22. Choose **create a new method**. A wizard pops up.
3. Choose **Next**. In the new window that pops up, select a configuration for instrument 1. Both the pump (9010, 9012, or ProStar230) and detector (9050, 9065, ProStar310, or ProStar330) modules should be listed on the right-hand side.
4. Click **Next**. Another window pops up. Make sure that the correct detector is listed.
5. Click on **Next**. A window pops up listing sections for post-run processing. Choose both data handling and standard reports.
6. Click on **Next**. A final window pops up. It should list the pump, detector, and both data handling and standard reports.
7. Click on **Finish**. A window pops up asking you to choose between opening a method or creating a new method.
8. Click on **Cancel**. The Method Builder screen with all the sections and subsections is displayed. Follow the instructions in "Setting Up Method Parameters" on page 48 to set up the method parameters.
9. When finished setting up the method, open the **File** menu and choose **Save As**. Enter a name and click on **Save**. Open the **File** menu again and select **Exit**.

Setting Up Method Parameters

1. Click on method parameters under the 9012 or ProStar230, as shown in Figure 24.

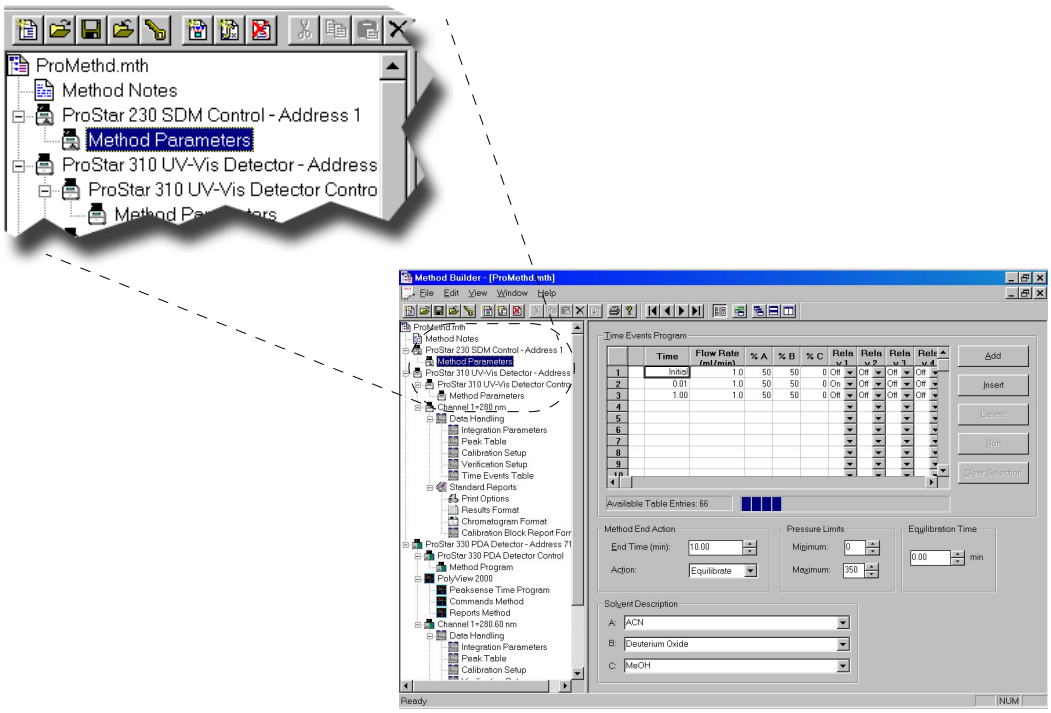


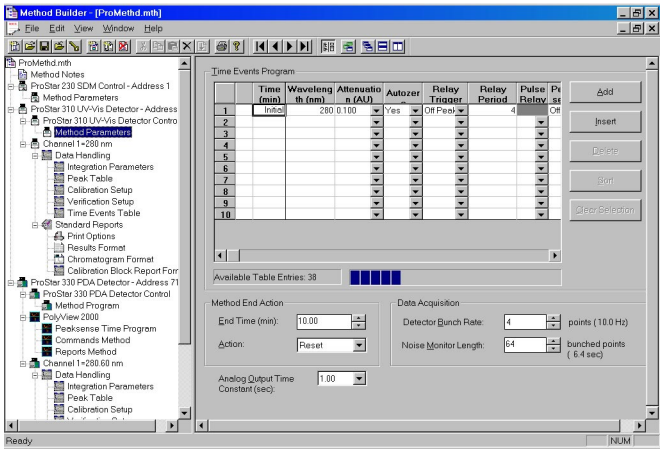
Figure 24. STAR Pump Method Window

2. Set up the pump method as needed. Figure 24 shows an example of a gradient method.
3. Move to the detector module click on Method Parameters.

   Figure 25 shows an examples of ProStar 310 and 330 methods.

4. To set the display wavelength in the ProStar 330, go to the peak sense time program option of Polyview 2000. select the desired wavelength. This should update the channel 1 heading.
5. To convert results files to ASCII in both the 9050/ ProStar 310, go to the Print Options page of Standard Reports. Select the Convert results to ASCII box. (Unselect the Automatic printing of reports & chromatograms if you don't want a STAR printout of each run or if you are not connected to the printer).

ProStar 310 Detector Method Window



STAR ProStar 330 Detector Method Window

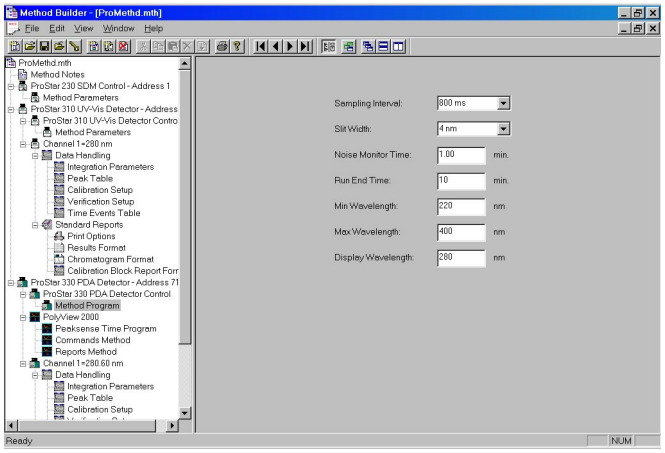


Figure 25. Detector Method Windows

6. To include a run log that has the pump and detector method parameters, go to the Results format page under Standard Reports and select the Run log box.
7. After you are finished entering parameters, select Save or Save As from the File menu.
8. Select Exit from the File menu.
9. If necessary, click on the System Control icon to return to the System Control window.
10. Open the file menu and chose activate method. A list of method files is displayed. Choose the correct method and click on **open**.
11. For the method to download to the pump, the pump must be running. Click on the **pump** button on the panel display.

3.7 Setting Up the Ethernet Connection

The Dell computer has a built in ethernet connection. Establishing communication with the network at the customer site is the responsibility of the customer. A network connection is highly recommended for data transfer from the PC to the Sun computer and for printing from the PC.

Chapter 4. LC-NMR Analyte Collector

Sections in this chapter:

- 4.1 “Connecting the Analyte Collector to the LC Workstation” this page
- 4.2 “Installing the Analyte Collector Plumbing” page 53
- 4.3 “Analyte Collector Flow Diagrams” page 54
- 4.4 “Setting Up Software for Analyte Collector” page 56

The optional LC-NMR Analyte Collector allows the collection and storage of up to 15 analytes (fractions) automatically (or 16 manually). The Analyte Collector can be operated manually with switches on the front panel or by software with the Stop-Flow Monitor program on the LC STAR workstation. Designed to trap analytes as they elute from an HPLC system, the Analyte Collector is capable of operating at moderate pressures and maintaining the analytes in the conditions under which they eluted. When connected directly to the NMR spectrometer, the Analyte Collector replaces the stop-flow valve in both hydraulic and electrical connections.

The Analyte Collector comprises a base module and a loop module, as shown in Figure 26.

- The base module contains two valves that control where the HPLC effluent is directed, the same as the stop-flow valve used to do.
- The loop module is a detachable, 16-port valve that can be moved between base units. The 16-port valve has 130 µL 0.01-inch I.D., 1/16-inch O.D. PEEK tubing loops installed. Larger or smaller loops can be installed.

4.1 Connecting the Analyte Collector to the LC Workstation

The Analyte Collector connects to the LC workstation with a terminal strip on the back of the base module. This general purpose device uses contact closure to change valve positions. Read back from the loop module occurs through BCD outputs.

To connect the Analyte Collector, make the connections shown in Table 3. Disconnect the stop-flow valve (P5) when using the Analyte Collector.

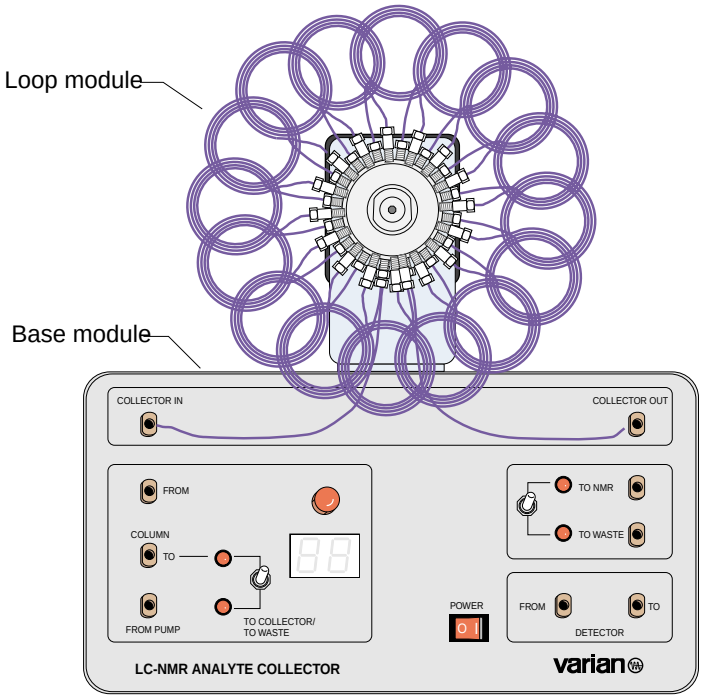


Figure 26. LC-NMR Analyte Collector

Table 3. Analyte Collector Wire Connections

Pin	*Function*	*Wire Color*
1	To Column (–)	white/green
2	To Column (+)	white/orange, or white only
3	To Collector/waste (–)	none
4	To Collector/waste (+)	white/yellow
5	To Waste (–)	none
6	To Waste (+)	gray
7	To NMR (–)	jumpered from pin 18
8	To NMR (+)	violet
9	Error NO	yellow
10	Error C	jumpered from pin 18
11	Step Input (+)	white/red
12	Step Input (–)	white/black white/brown
13	BCD 1.1 output	brown
14	BCD 1.2 output	red
15	BCD 1.4 output	orange
16	BCD 1.8 output	green
17	BCD 10.1 output	blue
18	Common	black

4.2 Installing the Analyte Collector Plumbing

The Analyte Collector replaces the stop-flow valve in the LC-NMR system. The procedure below describes how to install the plumbing for the base module of the Analyte Collector. Figure 27 is a diagram of the plumbing connections.

1. Turn off the LC pump, LC detector, and exit from the Stop Flow Monitor program.
2. Connect the outlet from the mixer to the **From Pump** connector.
3. Connect the inlet to the injector valve to the **To Column** connector.
4. Connect the outlet of the column to the **From Column** connector.
5. Connect the inlet of the detector to the **To Detector** connector.
6. Connect the outlet of the detector to the **From Detector** connector.
7. Connect the NMR transfer line to the **To NMR** connector.
8. Connect the **To Waste** connector to an approved waste container.
9. For stopped-flow or on-line runs, connect a tube between **Collector In** and **Collector Out**. Do not connect the loop module.
10. For analyte collection or analysis, connect the loop module to the appropriate **Collector In** and **Collector Out** connectors.

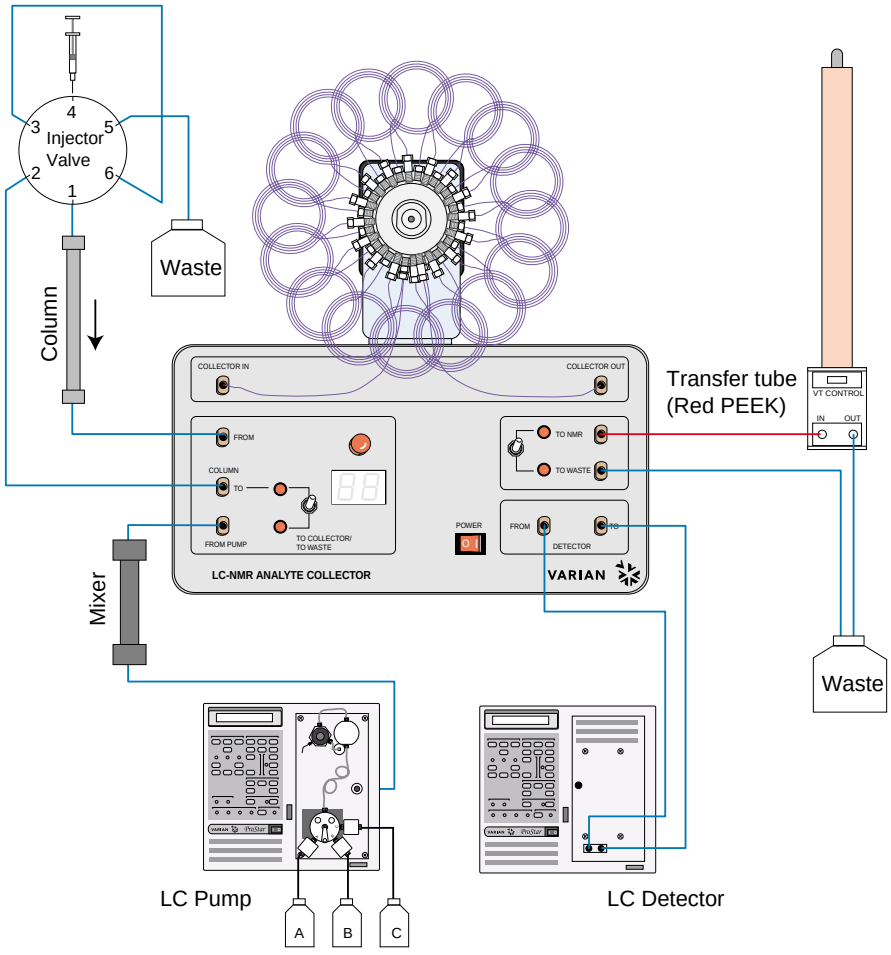


Figure 27. Analyte Collector Hydraulic Connections

4.3 Analyte Collector Flow Diagrams

This section contains the following Analyte Collector flow diagrams:

- Collecting separated peaks (Figure 28)
- On-flow (Figure 29)
- Stop-Flow—stopped (Figure 30)
- Loop elution (Figure 31)

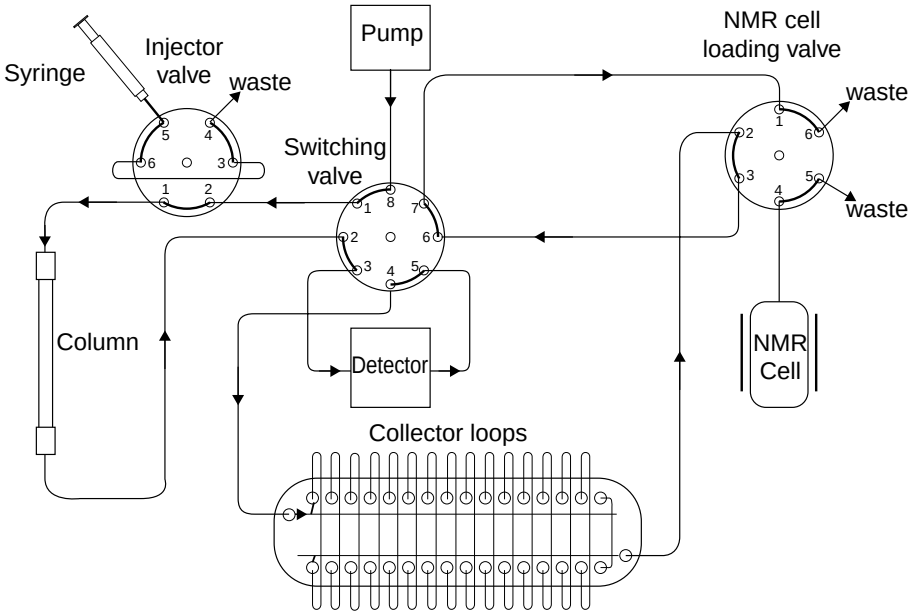


Figure 28. Flow Diagram for Collecting Separated Peaks

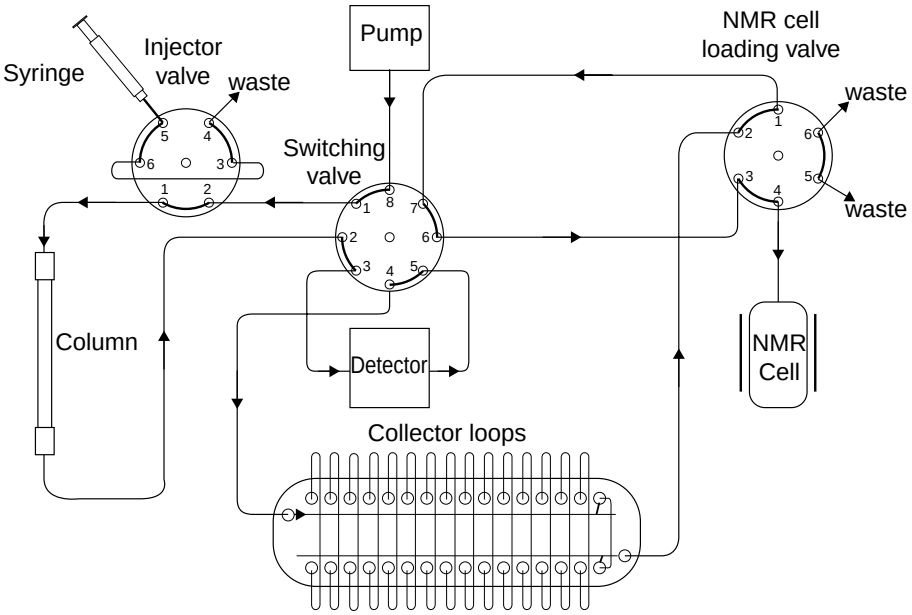


Figure 29. Flow Diagram for On-Flow

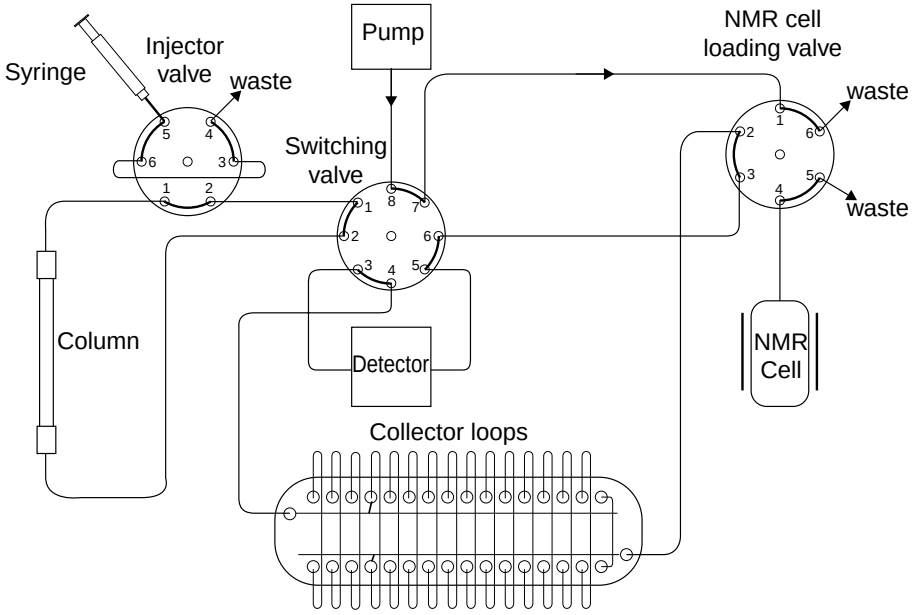


Figure 30. Flow Diagram for Stop-Flow—Stopped

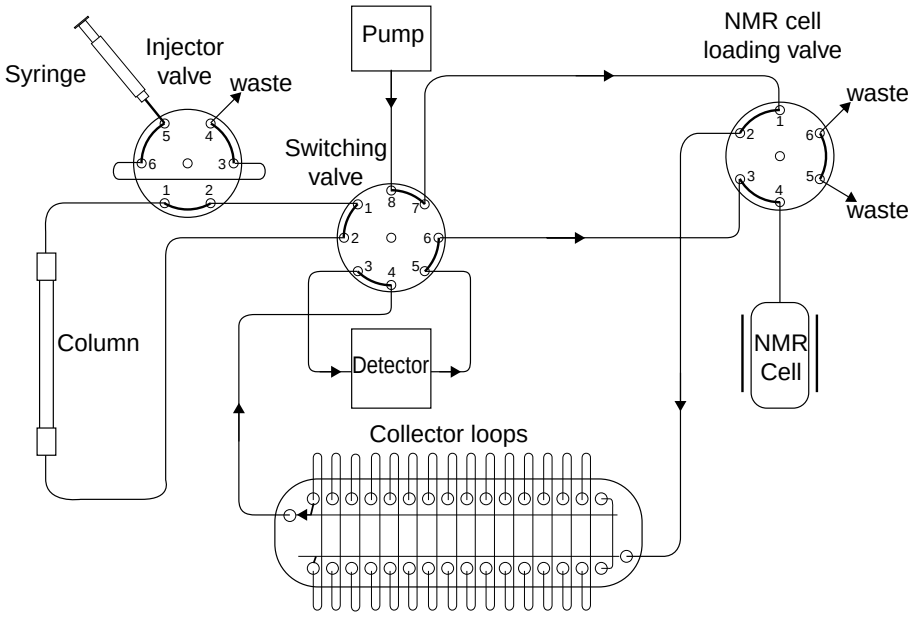


Figure 31. Flow Diagram for Loop Elution

4.4 Setting Up Software for Analyte Collector

1. In the Cascade program, open up the system configuration window, which is shown in Figure 32.
2. Click on the **analyte-collector on-line** box as shown in
3. Click on **Save as Default** and then close the window.

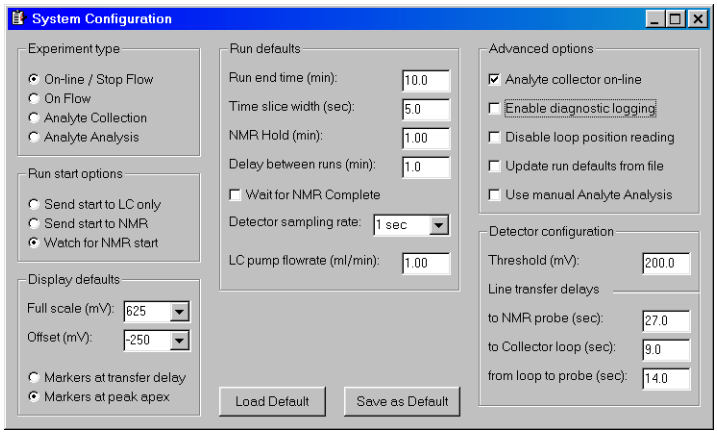


Figure 32. System Configuration Window

Chapter 5. LC System Calibrations

Sections in this chapter:

- 5.1 “Calibrating LC1D WET” this page
- 5.2 “13C Sideband Suppression” page 58
- 5.3 “Calibrating the Delay Time” page 58
- 5.4 “New Solvent Concentration” page 61

The LC hardware must be installed and connected, and the probe must be installed and tested before these calibrations can be done.

5.1 Calibrating LC1D WET

This procedure describes how to do the `lc1d` WET solvent suppression calibration. This procedure uses the LC1D pulse sequence and requires the 20% to 50% CH_3CN/D_2O sample.

1. Enter **`lc1d`** to create the parameters for the LC1D pulse sequence.
2. Enter **`setscout ga`**.

   The `setscout` macro turns off water suppression and lowers transmitter power. The `ga` command acquires a nonsuppressed spectrum on the solvent mixture.
3. Enter **`setwet`**.

   The `setwet` macro raises the transmitter power, places `tof` at the CH_3CN frequency, and sets the `wetshape` to solvent shift for HOD.
4. Enter **`ssfilter='n'`** to turn off solvent subtraction.
5. Enter **`array('wetpwr',20,-10,0.5) ga`** to create an array of `wetpwr` in 0.5-dB steps.
6. Enter **`dssh`** to display the spectra you just acquired.
7. Enter **`wetpwr=wetpwr[`*n*`]`**, where *n* is the number of spectra with the CH_3CN best suppression. This calibration should be checked occasionally, particularly if suppression deteriorates.
8. Enter **`gtw=0.002 gzlvlw=1000,2000,4000`** … **`30000 ga`** to run a gradient strength array.
9. Enter **`gzlvlw=gzlvlw[`*n*`] ga`** where *n* is the number of spectra with the best CH_3CN suppression and minimal artifacts. This number does not need to be recalibrated unless suppression or artifacts change significantly.
10. Enter **`ssfilter='y' wft`** to turn on solvent subtraction of CH_3CN.

5.2 ^{13}C Sideband Suppression

This procedure describes how to decouple ^{13}C sidebands during WET pulses.

1. Tune the X channel of the probe to ^{13}C connect the decoupler cable.
2. Enter **`dmfwet=427 dpwrwet=21`**.
3. Set **`dof`** to the approximate ^{13}C shift of CH_3CN.
4. Set **`dmm`** or **`dseq`** for GARP1 decoupling.

   CW mode is also acceptable.
5. Set **`c13wet`** to one of the following:
   - `c13wet='y'` eliminates ^{13}C sidebands. Data normally responds well to `ssfilter=80 ssntaps=161`.
   - `c13wet='n'` keeps ^{13}C sidebands. Data may look better with `ssfilter='n'`, because of phase distortion of ^{13}C sidebands.

5.3 Calibrating the Delay Time

Several delay times must be calculated: one for the stop-flow valve and three for the analyte collector.

For delay time calibrations a sample of only one of the benzoates is needed. Prepare a sample of the sample concentration 0.5 mg/ml (dilute with 50/50 acetonitrile/D_2O) and use 50 microliter injections.

Set up an isocratic method of 57% acetonitrile and 43% deuterium oxide.

Calibrating from the LC Detector to the Microflow Probe

The LC-NMR system can perform both continuous on-flow spectral measurements and stop-flow measurements. Continuous on-flow experiments do not require a delay time. A stop-flow measurement is typically used if the sample concentration is too low to be seen on-flow or if more extensive experiments such as COSY, TOCSY, or HMQC are required.

In order to do stop-flow, a detector more sensitive than the NMR is required to allow the LC peak of interest to be detected and stopped in the NMR probe. Between the detector and the NMR probe is a volume that is equal to the tubing connecting the two detectors, any intervening devices, and the volumes of the flow cells of the detector and the NMR probe. Since the LC system is forcing solvent through the system at a flow rate set by the operator, an LC peak requires a finite amount of time to travel from the detector to the NMR probe. This time, called the *delay time*, is calculated with the following procedure.

A typical delay time is 10–12 seconds using the stop-flow valve at a flow rate of 1 mL/min with 3 m (10 ft) of 0.005-in. I.D. tubing connecting the stop-flow valve to the flow probe. If the analyte collector is plumbed in, the delay time is on the order of 25-29 seconds at 1 mL/min.

1. Start the LC system and begin the Cascade LC-NMR program on the PC.

   If using p-hydroxy methyl benzoate, set up an LC method for 50% acetonitrile/50% D_2O; 1mL/min flowrate; 5 minute run time; and 280 nm detection.
2. In the **To NMR Probe** field, enter one of the following:
   - **12** seconds if using the stop-flow valve.

- **25** seconds if using the analyte collector.

3. Set **Hold Time** to 1 minute. Make sure **Wait for NMR** is **unchecked**.
4. Prepare a sample that provides an easily observable NMR peak under continuous on-flow conditions. You should be able to see a peak with `nt=4` or less.
5. Set up the NMR appropriately for LC-NMR. Set **`nt=4`** and set **`ni`** to a large value that allows you to observe at least one of the LC peaks that elutes off the column and through the probe. Expanding the region around where you expect to see an NMR peak is helpful; however, you can expand this region after data is collected.
6. Start the LC system. Start the NMR before the peak reaches the probe.
7. Monitor the NMR as the LC peak elutes through the probe. As the peak moves into the probe, the signal increases.

   If your calculated Delay Time is correct, you should see a profile similar to Figure 33 after you enter **`dssh`**.

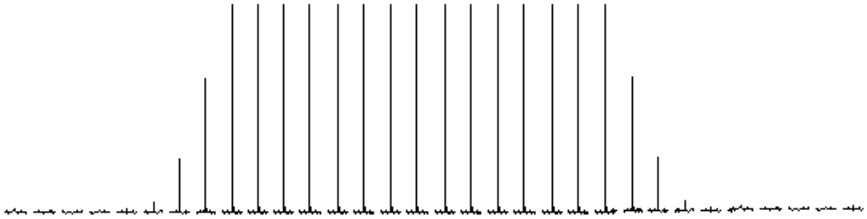


Figure 33. Profile Showing Delay Time is Correct

If the delay time is too short, the profile looks like Figure 34.

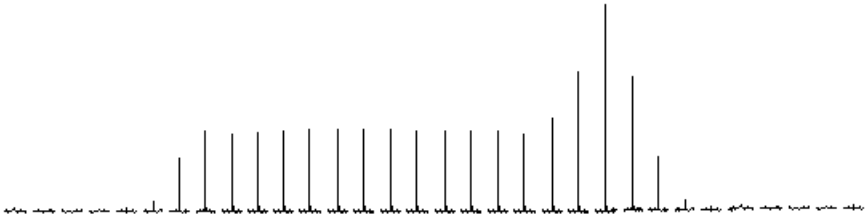


Figure 34. Profile Showing the Delay Time is Too Short

Fix this by increasing the delay time. You can estimate the amount of increase required from the time required from each spectrum and the number of spectra taken from when the valve closes to when the apex passes through the probe.

If the delay time is too long, the profile looks like Figure 35.

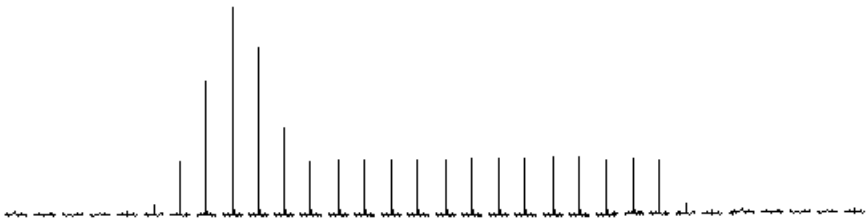


Figure 35. Profile Showing the Delay Time is Too Long

Fix this by decreasing the delay time.

Calibrating from the LC Detector to the Analyte Collector

The NMR system is not needed for calibrating from the LC detector to the analyte collector. It is best to use an isocratic run. Make sure that the end time of the 330 or 310 detectors matches the end time of the 230 pump.

1. Open the **System Configuration** window of the Cascade program.
   - Make sure that the **Analyte collector on-line** box is **selected**.
   - Select **Analyte Collector** for the **Experiment Type: On Flow/ Stop Flow**.
   - Make sure **Wait for NMR Complete** is **not selected**.
   - Enter **5 seconds** for **To Collector Loop**.
   - Check to see that the calibration is starting in loop 1.
   - Make sure the **Inhibit apex detection** is **not selected**.
2. Start a run with the benzoate sample. use a 50 µL injection equal to 25 mg. Use a 50% acetonitrile/50% D_2O method with 1mL/min flow rate. Set the detection wavelength to 280 nm.

   The first sample will be stored in loop 1 with the first loop fill delay time of 5 seconds.
3. Repeat steps 1 and 2 using different delay times, in one-sec increments, up to 11 seconds.
4. Store successive samples in successive loops. This process fills 7 loops. Record the apex intensity of the signal stored in each loop.
5. Do an analyze loops cycle.
   a. In STAR Chromatography, create an **isocratic method** of 50% acetonitrile/ 50% D_2O with a **2 minute run time**. Set the detector wavelength to 280 nm.
   b. Inhibit injection by not setting the relays in the pump section of the method.
   c. **Save the method** and then go to **System Control** and **Activate the method** and wait for the ready lights on the pump and detector.
   d. In the **System Configuration** window of Cascade, check **Analyte Analysis**.
   e. Set the **Run end time** to **2 minutes**.
   f. In the **Loop analysis** section of the Analyte Collector window of Cascade, set the start of analysis to **loop 1** and finish with loop 7. Set a **delay of 3 seconds** and a wait of 4 minutes.
6. In the **Main menu** (data acquisition and display) click on **Start Run**. Select **Inhibit apex detection**.
7. Each loop should be automatically emptied in turn and the UV spectrum recorded. Record the apex intensity of the eluted compounds. The correct delay time is the shortest time necessary for maximum intensity. Typical values at 1 mL/min are between 6–10 seconds. Enter the value in the configuration window of the LC-NMR stop-flow program in the To collector loop window.

Calibrating from the Analyte Collector to the Microflow Probe

Once the delay time from the UV detector to the analyte collector has been calibrated, it is necessary to calibrate the delay time from the analyte collector to the flow probe.

1. Load loops 1 to 10 with the methyl benzoate sample. This requires 10 separate injections.
   - Use an isocratic method: 50% acetonitrile/50% D_2O. 5-min. end time, 1 mL/min, 280 nm detection.
   - Inject 25 mg on-column (50 µL of 25 mg/50mL standard)
2. Set the system up for analyte analysis:
   a. In **System Control** of STAR Chromatography, activate the **LC method** used for analyte analysis in the previous calibration.
   b. In **System Configuration** of Cascade, select **Analyte Analysis**.
   c. Set the **hold time** to **1 minute** and make sure that the system is not waiting for the NMR to complete.
   d. Set the **Threshhold** to **200 mV**.
   e. Set the **End time** to **2 minutes**.
   f. Start with a **from loop to probe** delay set to **10 seconds**. This assumes that the LC method is at 1 mL/mi). Save the configuration.
   g. Make sure that the **Inhibit apex detection** is **not selected** on the main menu.
   h. Go to the analyze loops section in the analyte collector window, set the wait time between runs to 3 to 4 minutes. Start and end with loop 1. Close the analyte collector window.
   i. Click on **Start Run** in the main menu to start analyte analysis.

   After the wait time is counted down, loop 1 will be emptied and a peak should be detected in the LC/NMR stop-flow program after it crosses the threshhold. The delay time will be counted down and the system will stop while the NMR acquires data.
3. After the hold time has expired, the LC-NMR run will continue.
4. After the NMR and Cascade finish, change the **from loop to probe** delay time to 11 seconds in the **System Configuration** menu.
5. In the **Analyte Collector** window, set the first and last loop to be analyzed to 2.
6. When the LC pump and detector are ready, click **Start Run**. Loop 2 will be emptied, and the new delay time will be invoked after a peak is detected.
7. Increment the **from loop to probe** delay time for each subsequent loop and set up an analyte analysis run for that loop. The correct delay time is the one that results in NMR data as shown in Figure 33. Typical values are between 10 and 15 seconds at 1 mL/min.

Note: Changes in the length or I.D. of the connecting LC tubing will require the delay times to be recalibrated. Changes in flowrate will also affect the delay time, but new delay times can be calculated. For example, changing the flowrate by half results in a two-fold increase in the delay time.

5.4 New Solvent Concentration

For a new solvent concentration, enter the macro `getwet`. The `getwet` macro runs `setscout ga setwet ga`, which acquires an `nt=1` scout scan and a solvent-suppressed spectrum of the new solvent mixture.

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